PHARMACOLOGICAL STUDIES OF PROSTANOIDS AND OTHER SUBSTANCES ON SENSORY NERVES IN ARTHRITIC RATS

By

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ABSTRACT

A novel model of unilateral, localized adjuvant-induced arthritis in the rat was assessed for its use in pharmacological studies on the pain associated with chronic joint inflammation. Levels of inflammation, changes in behaviour and joint pathology were examined. Electrophysiological recordings of afferent activity were made in anaesthetized rats *in vivo* and *in vitro* from a fine branch of the medial plantar nerve innervating the tarsal joint. The effects of various putative inflammatory mediators on the discharge of articular nociceptors both in normal and arthritic joints were also examined. Where possible, selective agonists and antagonists were used to characterize the pharmacological receptors involved in the actions of these substances.

Injection of Freunds Complete Adjuvant around the left ankle joint caused a localized swelling, nociceptive thresholds to mechanical stimuli were lowered and use of the injected joint was significantly reduced. Histological studies revealed the presence of an arthritis which was confined to the injected ankle. Electrophysiological investigations showed that in arthritic joints there were a larger number of identifiable receptive fields for articular mechanonociceptors compared to those found in normal joints. Receptors had lower activation thresholds, and often displayed a resting discharge not seen in normal joints.

Intra-arterial (i.a.) injection of 5-hydroxytryptamine (5-HT), excited articular nociceptors from normal and arthritic joints. Responses consisted of two components: (a) a fast transient burst of activity mediated by a 5-HT_3-receptor, followed by (b) a delayed, longer-lasting excitation mediated by a 5-HT_2-receptor. These responses were shown to occur both in vivo and in vitro. 5-HT also increased the mechanical responsiveness of articular mechanonociceptors via a 5-HT_3-receptor. Sensory receptors in arthritic joints were more sensitive to 5-HT than those from normal joints. Administration of the 5-HT_3- or 5-HT_2-receptor antagonists caused short-lasting reductions in background activity in arthritic joints, as well as in normal joints in which activity had increased following administration of 5-HT.

In normal joints, i.a. injection of PGE_2, PGI_2 or the selective IP-receptor agonist cicaprost, excited and caused mechanical sensitization of articular mechanonociceptors. Potentiation of the short-lived excitatory and sensitizing effects of i.a. injected bradykinin on these receptors was also shown. Examination of the effects of PGE_2, PGI_2 and cicaprost in vivo or PGE_2, cicaprost, PGD_2, and PGF_2 alpha in vitro, produced a rank order of potency of

$$PGI_2 = \text{cicaprost} \gg PGE_2 \gg PGD_2 = PGF_2 \text{ alpha}$$

In arthritic rats injection of cicaprost, and to a lesser extent PGE_2, was effective in increasing the mechanical responsiveness and resting discharge of articular mechanonociceptors previously depressed by i.v. lysine acetylsalicylate. These results provide evidence for the involvement of IP-receptors, and perhaps EP-receptors, in the excitatory

and sensitizing actions of the prostanoids on articular nociceptors, and suggest that PGI_2 is the major endogenous prostanoid responsible for the mechanonociceptor sensitization seen in arthritic rat ankle joints.

Overall the results suggest that more than one mediator is required to produce conditions of mechanonociceptor sensitization seen in arthritic rat ankle joints. However, as modulators of the responsiveness of nociceptors to other mediators, and as potent excitants themselves, the prostanoids, and in particular PGI_2, probably play a major role in the alterations in nociceptor sensitivity seen in arthritic joints.

DECLARATION

I declare that this thesis was composed entirely by myself, and that the work on which it is based is my own with the following exceptions:

(i) Approximately half of the electrophysiological experiments studying the effects of 5-hyroxytryptamine, bradykinin, PGE_2, paracetamol and aspirin *in vivo* were carried out in collaboration with Dr B.D. Grubb or Dr D.S. McQueen. In such cases either surgery or injection of drugs was carried out by these persons.

(ii) Sectioning, mounting and staining of rat hindlimbs for histological studies was carried out by Mr Gordon Goodall.

(iii) Extraction of prostanoids from ankle joint tissues was carried out in collaboration with Miss Heather Mather and prostanoid radioimmunoassys were carried out with further assistance from Mrs Lorna Turnbull.

(iv) Colorimetric determination of plasma salicylate levels was carried out by Mr Alistair Macdonald, and HPLC estimation of plasma paracetamol levels was carried out by members of the Department of Therapeutics and Clinical Pharmacology, The Royal Infirmary, Edinburgh.

I contributed substantially to the design of all experiments, and to the analysis and subsequent interpretation of results.

G.J. Birrell

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PUBLICATIONS

Results from some of the experiments reported in this thesis have been published (see Appendix II) or are awaiting publication as follows:

Birrell G.J., Grubb B.D., Iggo A. and McQueen D.S. (1990) Actions of PGE2 and cicaprost on the sensitivity of high threshold mechanoreceptors in normal and inflamed rat ankle joints. J.Physiol. 420: 33P

Birrell G.J., Grubb B.D., Iggo A. and McQueen D.S. (1990) Sensitization of high threshold mechanoreceptors by 5-hydroxytryptamine (5-HT) in anaesthetized normal and arthritic rats. J.Physiol. 420: 34P

Birrrel G.J., McQueen D.S., Iggo A. and Grubb B.D. (1990) Comparison of the effects of PGE2 and cicaprost on the responses of rat ankle joint mechanoreceptors to bradykinin. Pain Suppl. 5: S135.

Birrrel G.J., McQueen D.S. and Iggo A. (1990) Comparison of the effects of PGE2 and cicaprost on the responses of rat ankle joint sensory receptors to bradykinin in vitro. Proceedings of Satellite Meeting to the VIth World Congress on Pain: The Neurobiology of Nociception. Kauai: P2.

Birrrel G.J., McQueen D.S., Iggo A. and Grubb B.D. (1990) The effects of 5-HT on articular sensory receptors in normal and arthritic rats. Br.J.Pharmacol. 101: 715-721.

Birrell G.J., McQueen D.S. and Iggo A. (1990) Effects of cicaprost and PGE2 on the responses of rat ankle joint sensory receptors to bradykinin in vitro. J.Physiol. 429: 29P.

Birrell et al. (1990) PGI2-induced activation and sensitization of articular mechanonociceptors. Neurosci. Lett. In Press.

Grubb B.D., Iggo A., McQueen D.S. and Birrell G.J. (1989) The effects of prostaglandin E2 and bradykinin on the discharge of articular mechanoreceptors in normal rats. Proceedings of the XXXI International Congress of Physiological Sciences. Helsinki.

Grubb B.D. Birrell G.J., McQueen D.S. and Iggo A. (1990) The role of PGE2 in the sensitization of mechanoreceptors in normal and inflamed ankle joints of the rat. Exp.Brain Res. In Press.

Grubb B.D., McQueen D.S., Iggo A. and Birrell G.J. (1988) A study of 5-HT receptors associated with afferent nerves located in normal and inflamed rat ankle joints. Agents and Actions 25: 216-218.

McQueen D.S., Iggo A., Birrel G.J. and Grubb B.D. (1990) Effects of aspirin and paracetamol on high threshold tarsal joint mechanoreceptors in anaesthetized rats with adjuvant-induced arthritis. J.Physiol. 425: 35P.

Smith G.D., Bowden A.F. Birrell G.J. McQueen D.S. and Harmer A.J. (1990) Neuropeptide content of dorsal root ganglia cells in the arthritic rat. J.Physiol. 430: 112P.

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LIST OF ABBREVIATIONS AND UNITS

ASA	acetylsalicylic acid
BK	bradykinin
C	carbon
^{o}C	degrees centigrade
Ca^{2+}	calcium cation
cAMP	cyclic adenosine monophosphate
CGRP	calcitonin gene related peptide
C.N.S.	central nervous system
CO2	carbon dioxide
delta	change in
delta max	change in maximum
delta $\bar{x}$	change in the mean, also mean difference
delta $\bar{x}$%	change in mean expressed as a percentage change
delta Σx	discharge integrated with respect to control
ED50	dose causing 50% maximum response
g	gram
H^{+}	hydrogen ion
HCl	hydrochloric acid
5-HT	5-hydroxytryptamine
i.a	intra-arterial
i.p.s.	impulses per second
i.v.	intra-venous
K^{+}	potassium ion
kg	kilogram
Krebs	Krebs-Heinsleit solution (modified)
l-AS	lysine acetylsalicylate
log	logarithm
LT	leukotriene
M	molar concentration
max	maximum
mg	milligram
$mgkg^{-1}$	milligrams per kilogram
μg	microgram
min(s)	minute(s)
ml	millilitre
ml/min	millilitres per minute
mm	millimetre
μm	micrometre
mV	millivolt
n	number of observations

Na^+	sodium cation
NaCl	sodium chloride
NSAID	non-steroidal anti-inflammatory drug
O_2	oxygen
P	statistical probability
PG	prostaglandin
s	second
s.e.m.	standard error mean
Σx	total counts
t	time
Tris	Tri(hydroxymethyl)aminomethane
TX	thromboxane
w/v	weight per volume
$\bar{x}$	arithmetic mean

Most abbreviations, other than commonly used expressions, are also defined at the first point of occurence in the text.

INDEX TO CONTENTS

SECTION I

GENERAL INTRODUCTION

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GENERAL INTRODUCTION

1.1 Introduction

The severe chronic pain experienced by individuals affected by arthritis or rheumatism is the major clinical symptom associated with this class of disorders. The most effective treatment available is the administration of non-steroidal anti-inflammatory drugs (NSAIDS) such as aspirin (Hart, 1987). However, NSAIDS are known to cause moderate to severe damage of the gastrointestinal mucosa (Goodwin, 1987), damaging effects on renal function (Lifschitz, 1983), and a wide range of less common side effects. In addition, these drugs may potentiate chronic inflammation (Lewis & Barrett, 1986), and have a harmful effect on cartilage, especially on that already compromised by disease (Palmoski & Brandt, 1985). Opioid analgesics are often used in conjunction with NSAIDS, but are generally of little additional benefit (Cooper & Beaver, 1976; Ehrnebo et al., 1977; Findlay et al., 1978), and at higher doses cause centrally-mediated side effects such as drowsiness, dizziness, nausea, and vomiting. In order to develop new drugs for the treatment of arthritic pain it is essential that we understand the mechanisms responsible for its production. This requires in depth knowledge of the physiological systems involved in the processing of stimuli perceived as painful, and, in addition, it is important that we understand the

changes which take place during inflammation which cause alterations in sensory sensitivity. My aim in the General Introduction will be to outline the physiology of the peripheral origins of pain, and go on to examine the role played by these mechanisms in the pain associated with arthritis.

1.2 Pain and nociception

Early observations from the work of Blix (1884) and Von Frey (1895) provided evidence for the existence of specific receptors in the skin that were capable of responding to stimuli perceived as painful. By the turn of the century, researchers encountered the problem of investigating pain in animals which could not report whether a stimulus was painful or not. Following the work of Sherrington (1906), the term nociception was used to describe the sensory sub-modality responding to those stimuli defined as "capable of compromising the integrity of the organism". In animals these stimuli produce reflex responses such as flexion reflex, increased heart and respiratory rates, and pupilary dilatation. In awake animals accompanying behavioural responses such as escape reactions and vocalisation also occur.

The discovery that different classes of nerve fibre exist in the periphery led to the demonstration, using graded electrical nerve stimulation, that nociceptive input is transmitted by small myelinated (A-delta: corresponding to the "B wave" of the compound action potential) and unmyelinated (C: corresponding to the "C wave") afferent axons. The classical studies, using partial nerve blocking techniques, conducted by Gasser & Erlanger (1929) and later by Clark et al. (1935)

confirmed these findings and further showed that A-delta fibres were required for sharp, well localised pain sensations, whereas C fibres were involved in a delayed, more diffuse pain, often of a burning type.

The first electrophysiological recordings of the activity of afferent nerve fibres indicated that small diameter nerve fibres could be excited by different types of noxious stimuli (Adrian, 1931; Zotterman, 1939). However, these and subsequent investigators found fine fibres which were also activated by a range of innocuous stimuli. It was not until the late 1960s that enough evidence had been collected to declare the existence of well-defined specific "nociceptors" in the skin (see Burgess & Perl, 1973).

Due to the ease of access to the cutaneous tissues in animals and particularly in man, nociceptors in these tissues have been studied in greatest detail. Nociceptors present in the skin possess similar characteristics to those found in other tissues (Besson & Chaouch, 1987), and for this reason, I will devote the next major section to a description of their identifying features.

1.3 Classification of cutaneous nociceptors

Work carried out mostly during the 1960s in cats, dogs, rats and primates (Iggo, 1958,1959; Hunt & MacIntyre, 1960; Iruichijima & Zotterman, 1960; Witt & Griffin, 1962; Burgess & Perl, 1967; Perl, 1968; Bessou & Perl, 1969) identified two major groups of nociceptors (Burgess & Perl, 1973).

C-polymodal nociceptors: activated by strong stimuli, mechanical, thermal or chemical.

A-delta mechanonociceptors: activated by strong mechanical stimuli but unresponsive to chemical or thermal stimuli.

However, this clear distinction between A-delta and C fibre associated nociceptors was not apparent in subsequent studies using rats (Necker & Hellon, 1978; Kenins, 1981; Lynn & Carpenter, 1982; Fleischer et al., 1983) cats (Beck et al., 1974; Fitzgerald & Lynn, 1977), monkeys (Iggo & Ogawa, 1971; Beitel & Dubner, 1976a,b; Croze et al., 1976; Georgopoulos, 1976; Kumazawa & Perl, 1977; Lamotte & Campbell, 1978; Campbell et al., 1979; Meyer & Campbell, 1981) and humans (Torebjork & Hallin, 1972; Van Hees & Gybels, 1972; Torebjork, 1974; Torebjork & Hallin, 1974; Adriaensen et al., 1980; Konietzny et al., 1981; Adriaensen et al., 1983; Adriaensen et al., 1984). A great deal of heterogeneity exists within populations of nociceptors with A-delta or C fibre afferents. Thus some A-delta fibres in the cat or monkey can be activated by mechanical nociceptive and thermal nociceptive stimuli (Beck et al., 1974; Georgopoulos, 1976), and may function as polymodal nociceptors in man (Adriaensen et al., 1980). Furthermore, following application of a noxious thermal stimulus, an A-delta mechanoreceptor may respond to subsequent heat stimuli (Fitzgerald & Lynn, 1977; Campbell et al., 1979). Not all A-delta and C fibre afferents are nociceptive (Burgess & Perl, 1973), and conversely some fast conducting myelinated fibres are (Georgopoulos, 1976; Fitzgerald & Lynn, 1977; Campbell et al., 1979).

The response characteristics of all cutaneous nociceptors identified at this time defined a nociceptor as

(i) having response thresholds higher than that of low threshold mechanoreceptors or thermoceptors

and (ii) possessing the ability to code the intensity of nociceptive stimuli.

The following is a consideration of the main classes of cutaneous nociceptors identified and their response characteristics which enabled the above conclusions to be reached.

1.3.1 Characteristics of cutaneous mechanonociceptors

Cutaneous high threshold mechanoreceptors are mostly associated with A-delta afferent fibres and are not excited by thermal or chemical stimuli (Burgess & Perl, 1967). These receptors do not display any spontaneous activity, their rate of discharge increases with the stimulus amplitude, and they adapt slowly to a stimulus of constant intensity (Fitzgerald & Lynn, 1977). Receptive fields are usually made up of several sensitive zones, and application of repetitive stimuli to the same point in a receptive field has been reported to result in a characteristic fatigue in responsiveness (Burgess & Perl, 1967; Perl, 1968; Lynn & Carpenter, 1982).

1.3.2 Characteristics of cutaneous polymodal nociceptors

The characteristics of C polymodal nociceptors and A-delta polymodal nociceptors are, generally speaking, very similar (Bessou & Perl, 1969; Iggo & Ogawa, 1971; Beck et al 1974; Dubner et al., 1976; Georgopoulos, 1976; Price et al., 1977; Adriaensen et al., 1980). Receptive fields are usually punctate, often less than 0.5 mm diameter, although more complex receptive fields have also been identified (Beitel & Dubner, 1976a; Croze et al., 1976; Torebjork, 1979; Van Hees & Gybels, 1981), and a degree of overlap may exist between receptive fields of several afferent fibres (Torebjork, 1979). Under normal conditions polymodal nociceptors do not display any spontaneous activity.

Intense mechanical stimuli are required to produce excitation of polymodal nociceptors, and stimuli applied using pointed objects are the most effective. Discharge increases with the intensity of the applied stimulus and adapts slowly during a stimulus of fixed amplitude (Adrian & Zotterman, 1926). The development of fatigue following repeated mechanical stimulation is another characteristic of these receptors (Van Hees & Gybels, 1972; Torebjork & Hallin, 1974; Beitel & Dubner, 1976b; Kumazawa & Perl, 1977).

Skin temperatures of 40°C and above produce graded increases in the discharge of cutaneous polymodal nociceptors. The threshold for activation is generally around 42°C, although some receptors only fire at much greater temperatures (Bessou & Perl, 1969). Saturation of receptor discharge has been reported to occur at temperatures above 50°C (Beitel & Dubner, 1976a,b; Croze et al., 1976).

An outstanding feature of the response of polymodal nociceptors to

repeated thermal stimuli is the development of a marked sensitization. This results in several changes including a reduction in threshold to below 40°C, an increase in the response to a standard thermal stimulus, a reduction in the response latency and the appearance of spontaneous activity. (Witt & Griffin, 1962; Bessou & Perl, 1969; Beck et al., 1974; Beitel & Dubner, 1976a,b; Croze et al., 1976; Kumazawa & Perl, 1977; Lynn, 1979; Lynn & Carpenter, 1982; Lamotte et al., 1982; Fleischer et al., 1983) Heat-sensitized receptors have also been shown to have enhanced responsiveness to mechanical stimuli (Bessou & Perl, 1969). It should also be noted that exposure to intense heat (greater than 55°C), or application of repetitive stimuli, produces a marked desensitization of receptor responsiveness (Bessou & Perl, 1969; Beitel & Dubner, 1976b; Georgopoulos, 1976).

Although examination of the effects of chemical stimuli on cutaneous receptors has not been carried out routinely, several studies considering the responses of A-delta and C polymodal nociceptors to the cutaneous application of algogenic substances have been carried out in animals (Bessou & Perl, 1969; Forster & Ramage, 1981; Kenins, 1981; Lynn & Carpenter, 1982) and humans (Torebjork & Hallin, 1974; Adriaensen et al., 1980; Van Hees & Gybels, 1981; Adriaensen et al., 1983). These receptors are excited by the application of acids, bradykinin, histamine and other irritants to their receptive fields, and a good correlation exists between evoked activity and the reported pain sensation. Much work has examined the effects of intra-arterial injection of algogenic substances on cutaneous nociceptor activity. Results from these studies will be considered in later sections dealing with individual mediators and their effects on nociceptor function.

1.4 Cutaneous nociceptors and sensation

In general, cutaneous nociceptors are only activated by stimuli capable of causing tissue damage and which are perceived as painful when applied to humans. However, this is not always the case as nociceptors can respond at lower intensities of stimuli perceived as non-painful (Van Hees & Gybels, 1972; Torebjork & Hallin, 1974; Beitel & Dubner, 1976a). Application of mechanical or thermal stimuli which activate polymodal nociceptors do not always cause painful sensations (Torebjork & Hallin, 1972; Van Hees & Gybels, 1972). However, higher levels of activity from these same nociceptors produce pain, and there is a good correlation between the discharge frequency of nociceptors and the sensation of pain (Torebjork, 1979; Van Hees & Gybels, 1981; Torebjork et al., 1984). These observations suggest a role for temporal and spatial summation in coding of nociceptive input from the periphery.

Sensitization of nociceptors in both animals and humans provides an explanation for the hyperalgesia and hyperaesthesia caused by nociceptor stimulation, and these phenomena have proven to be extremely important, particulary in relation to the effects of tissue damage and inflammation on nociceptor function (see Besson & Chaouch, 1987).

1.5 Morphological studies of cutaneous nociceptors

Since the work of Von Frey (1895) the morphological correlate of the nociceptor has been regarded as the "free nerve ending". The term originally arose from work carried out during the nineteenth century leading to the observation that unmyelinated fibres do not acquire a

corpuscular or specialized encapulated structure at their peripheral ends, nor are they associated with any cells thought to participate in sensory processes (eg Merkel cells). Since these unmyelinated free terminals were often found to be the predominant or only morphological structure present in areas from which sensations of pain could be elicited, it was accepted that they were indeed nociceptors. More recent electronmicroscopic examination of serial sections from cutaneous tissues containing unmyelinated or fine myelinated terminals have revealed the more detailed structure of these endings (Cauna, 1973; Kruger et al., 1981). As they pass through the epidermis, terminals often remain enclosed within a Schwann cell process after loss of their myelin sheath. The terminals contain a mixed population of vesicles and also mitochondria, this arrangement being reminiscent of a synaptic bouton.

It seems likely that at the morphological level little differentiation between nociceptive terminals will be achieved. However, immunohistochemical studies examining predominantly the peptide contents of sensory terminals have revealed a previously unsuspected diversity and heterogeneity of the free nerve ending (see Kruger, 1987).

1.6 Nociceptors in tissues other than the skin

Sensory receptors with finely myelinated or unmyelinated afferent fibres have been identified in many different tissues. These receptors have been tested using a variety of stimuli, and have been found to possess similar response characteristics to those of cutaneous nociceptors. In the following sections, a brief outline of the putative nociceptor

populations in different tissues will be given. A separate section will be devoted to sensory receptors associated with articular tissue.

Visceral receptors with nociceptive properties have been identified in the abdomen (Zimmerman, 1979; Mei, 1983), colon (Blumberg et al., 1983; Haupt et al., 1983; Longhurst et al., 1984), bile duct (Cervero, 1982), reproductive system (Floyd & Morrison, 1974; Clifton et al., 1976; Floyd et al., 1976; Coggeshall & Ito, 1977; Kumazawaa and Mizumura, 1977b; 1980a,b), urinary system (Denny-Brown & Robertson, 1933; Iggo, 1955; Todd, 1964; Astrom & Crafoord, 1968; Beacham & Kunze, 1969; Niijima, 1971; Clifton et al., 1976; Floyd et.al., 1976), myocardium (White, 1959; Brown, 1967; Uchida & Murao, 1975; Nishi et al., 1977; Barker et.al., 1980), and respiratory system (Fillenz & Widicombe, 1972; Paintal, 1973). These receptors respond to a variety of stimuli, including stretch of the tissues, application of chemical irritants or tissue ischemia.

Receptors with A-delta afferent fibres which respond to strong torsion of a tooth have been identified in the periodontium (Mei et al., 1977), and tooth pulp A-delta and C fibre polymodal nociceptors have also been characterized (Olgart, 1974; Horiuchi & Mathews, 1976; Kollman et al., 1982). In the cornea, polymodal nociceptors with finely myelinated axons have been characterized which respond to corneal stroking, heating, acids, or cooling (Belmonte & Giraldez, 1981).

Receptors in muscle, with A-delta or C fibre afferents, have long been known to be excited by a variety of stimuli including strong mechanical or thermal stimulation and injection of hypertonic saline (Bessou & Laporte, 1958; Paintal, 1960; Bessou & Laporte, 1961; Iggo, 1961). Receptors with A-delta and C fibre afferents have been shown to have

very similar characteristics (Mense & Meyer, 1985). About half of the high threshold mechanoreceptors with either A-delta and C fibre afferents are also excited by bradykinin (Franz & Mense, 1975; Mense, 1977). In addition, a number of C fibre afferents are excited exclusively by chemical stimulation. Finally noxious thermal stimulation has been shown to excite a number of A-delta and C fibre associated receptors, further characterizing them as polymodal nociceptors (Kumazawa & Mizumura, 1976; 1977a).

1.7 Articular sensory receptors

The tissues of the joints are extensively innervated by both myelinated and unmyelinated sensory nerves, and the presence of nerve endings in the joint capsule has been known for over a hundred years. Investigations carried out mainly during the 1950s and 1960s identified a number of morphologically distinct sensory endings within the joint tissues of animals (Samuel, 1952; Andrew & Dodt, 1953; Hromada & Polacek, 1958; Polacek, 1966; Freeman & Wyke, 1967) and man (Gardner, 1950). On the basis of these studies articular sensory endings have been classified into four main types (Freeman and Wyke, 1967):

type (i) globular corpuscle: encapsulated arborising terminals (Ruffini-type corpuscle)

type (ii) cylindrical corpuscle: thick laminated capsule (Pacinian-type corpuscle)

type (iii) fusiform corpuscle: thin capsule and densely arborizing terminal (Golgi-Mazzoni-type corpuscle)

type (iv) free nerve ending: unmyelinated free terminal.

Ruffini-type endings are normally linked in clusters of three to six corpuscles, and are associated with small diameter myelinated axons. The functional receptor unit is formed by the cluster of corpuscles each of which is innervated by the same afferent nerve fibre. These endings are found predominantly in the peripheral layers of the joint capsule, with smaller numbers located on extrinsic ligaments and in para-articular periosteum and related tendons (see Freeman & Wyke, 1967).

Cylindrical corpuscles of the Pacinian type are innervated by myelinated afferent axons and are found individually or in clusters of two to four corpuscles. Receptor units are associated with a single axon which branches to supply each corpuscle with a single thickened terminal. Pacinian type corpuscles are found in the capsular tissue, predominantly in the deeper layers in apposition with the fibro-adipose subsynovial tissue. The articular fat pads, where they are attached to the joint capsule, also contain small numbers of these endings (see Freeman & Wyke, 1967).

The fusiform corpuscles are entirely absent from the joint capsule, and are found only in the joint ligaments. Innervated by a single large myelinated axon, each corpuscle contains many densely arborizing filaments which arise from the main axon within the end-organ itself. Clusters of two to three corpuscles may occur, these being supplied by the same axon, and being smaller in size than single corpuscles (see

Freeman & Wyke, 1967).

As in other tissues the free nerve endings consist of relatively undifferentiated non-corpuscular terminations composed of unmyelinated nerve filaments. They appear to exist as either closely meshed networks or as isolated nerve endings. Unmyelinated nerve networks are distributed throughout the fibrous capsule, adjacent periosteum, articular fat pads and in the adventitial sheaths of the articular blood vessels. Isolated free nerve endings arise from the terminal branching of unmyelinated or finely myelinated afferent axons. They are most numerous in the joint ligaments, in the tendons related to the joint capsule and in the deeper layers of the capsule. Free nerve endings of any type are not seen in the synovial tissues or the menisci (see Freeman & Wyke, 1967).

Until recently, electrophysiological studies examining the response characteristics of articular sensory receptors have focused mainly on those receptors with rapidly conducting afferent fibres (Andrew & Dodt, 1953; Boyd & Roberts, 1953; Boyd, 1954; Skoglund, 1956; Eklund and Skoglund, 1960; Burgess & Clark, 1969; McCall et al., 1974; Clark, 1975; Clark & Burgess, 1975; Grigg, 1975; Grigg, 1976; Grigg & Greenspan, 1977; Ferrell, 1980; Grigg & Hoffman, 1982; Grigg et al. 1982). The Ruffini-type endings appear to be excited by flexion, extension, direct pressure or stretch of the joint capsule, and are slowly-adapting (Boyd, 1954; Eklund & Skoglund, 1960; Burgess & Clark, 1969; Grigg & Hoffman, 1982). Golgi-Mazzoni endings are activated by flexion or by pressure applied to the ligaments or inside surface of the capsule and also show a slowly-adapting response (Burgess & Clark, 1969; Grigg & Hoffman, 1982). Rapidly adapting responses are displayed by the Pacinian

corpuscle-like receptors which are excited only during joint movements (Burgess & Clark, 1969).

From the few units with slowly conducting (A-delta) afferents described in the studies above, they are stated as being characteristically insensitive to joint flexion, extension or rotation and pressure on the capsule. It was thus concluded that the slowly conducting myelinated fibres were most likely to be associated with articular nociceptors (Burgess & Clark, 1969).

1.7.1 Articular nociceptors

It is particularly notable that the sensory afferent innervation of the joints is composed mainly of finely myelinated and unmyelinated nerve fibres (Langford, 1983; Langford & Schmidt, 1983; Guilbaud et al., 1985). Identification of the functional role played by the receptors associated with these afferents is therefore extremely important. Investigations carried out by Schaible & Schmidt (1983a,b) have resulted in the identification of a population of A-delta and C fibre units (termed by these workers as group III and group IV afferents respectively) which appear to function as nociceptors in the cat knee joint. The receptive fields for these units are located in the joint capsule and consist of one, or occasionally up to four, small (< 2 mm diameter) responsive zones. Both A-delta and C fibre units have high Von Frey thresholds ranging from 3 to 225 mN, although the latter are generally less sensitive, and respond in a graded manner over a wide stimulus range. The majority of these units were only activated by joint movements outside the normal working range, responses being largest when

extreme rotations of the tibia are executed. A few units either responded to nonnoxious movements (21%) or did not repond even to extreme joint rotation (34%). Finally, resting activity was found to be present in a number of instances for each of the different receptor types described above.

In the capsular tissues of the rat ankle joint a population of high threshold C mechanoreceptors with nociceptive properties have also been identified by Guilbaud et al. (1985). Slowly adapting mechanoreceptors with punctate (1 - 3 mm diameter) receptive fields were found to have Von Frey thresholds from 4.6 to over 80 mN, with over half of the units having thresholds greater than 80mN. All units possessed similar characteristics to those of cutaneous nociceptors in that they were able to code the intensity of the applied stimulus, developed fatigue following repeated mechanical stimuli, and displayed an absence of any resting discharge over the normal range of joint position. Light pressure on the ankle joint or small degrees of flexion or extension failed to produce activation. An insensitivity to mild thermal stimuli was also demonstrated for these receptors.

From these studies it is clear that A-delta and C fibre articular mechanoreceptors are involved in deep pressure sensation and nociception. Under normal circumstances these receptors signal that the joint is under damaging or potentially damaging stress.

1.8 Mediators of inflammation

The association between inflammation and disease, first described by Hyppocrates (460 - 377 BC), was accompanied by the assumption that an irritant (materia peccans) causes local disturbance of the body fluids, which attracts digestive materials responsible for catabolism and dissolution of damaged tissue. From the observations of Celsus (30 BC - 30 AD) on the cardinal signs of inflammation, calor (heat), rubor (redness), tumor (swelling) and dolor (pain), it was realized that these effects were probably produced by endogenous substances which were released following injury.

In the early nineteenth century it was suggested that inflammation resulted from the reflex activation of neuronal elements (Henle, 1846). This concept was later replaced by a predominantly vascular theory of inflammation (Cohenheim, 1873). The inflammatory elements were considered to be blood cells which invaded the tissues following their passage through a damaged vessel wall (Arnold, 1875; Weigert, 1889).

Not until the introduction of chemical techniques was the importance of increased concentrations of small molecules considered in inflammation (Schade, 1923). The discovery that histamine, synthesized by Windaus and Vogt (1907) or extracted from biological materials (Ackerman, 1910; Kutscher, 1910) or ergot preparations (Barger & Dale, 1910), could elicit an inflammation-like reaction in human skin (Eppinger, 1913), led Lewis (1927) to suggest that a group of 'H'-substances were the ubiquitous mediators of inflammation. However, potent antihistamines turned out to be of only limited therapeutic value in the treatment of inflammation (Bovet & Staub, 1937). Following these discoveries a series of criteria to be fulfilled by any putative

mediator of inflammation were formulated by Dale (1929):

(i) Reduction in the release of the substance or inactivation of the substance by known anti-inflammatory drugs

(ii) Induction by the putative mediator of some or all signs of inflammation

(iii) Release of the proposed mediator during an inflammatory reaction.

With the definition of these criteria, a more directed and critical approach to the identification of potential mediators was now possible. Menkin (1940) was the first to identify a series of polypeptide mediators, and suggested the existence of substances specific for eliciting each symptom of inflammation. This new approach, and the conviction that more than one inflammatory mediator existed, led to the description of a variety of mediators such as bradykinin (Werle & Berek, 1948; Rocha e Silva et al., 1949) serotonin (Rowly & Benditt, 1956), complement factors (Bloch et al., 1963), prostaglandins (Horton, 1969) and other derivatives of polyunsaturated fatty acids (Samuelsson, 1981) and phospholipids (Vargaftig & Benveniste, 1983).

1.9 Inflammatory mediators and pain

Pain and hyperalgesia are among the principal features of inflammation whether in its acute or chronic form. Hyperalgesia and inflammatory pain

are thought to result predominantly from the actions of various chemical mediators on nociceptive endings within the affected tissues.

One of the first studies suggesting the action of an endogenous chemical mediator was carried out by Lewis (1942), who examined the pain-producing effects of ciculatory occlusion on active muscle. This observation was attributed to the effects of a 'factor P' which was later shown not to be lactic acid, CO2 or acidity (Dorpat and Holmes, 1955). Induction of pain by putative inflammatory mediators has long been a subject of investigation (Feldberg, 1956), but it was the studies of Keele & colleagues that thoroughly established their pain producing properties (Armstrong et al, 1952; Armstrong et al., 1953; Armstrong et al., 1957; Keele and Armstrong, 1964). Keele and his coworkers demonstrated that application of algogens to the exposed cantharidin-induced blister base induced painful sensations in man. Among the substances found to be specific algogens were acetylcholine, 5-HT, histamine, substance P and bradykinin.

Hyperalgesia has been considered for some time to result from the chemically-induced sensitization of nociceptors in the periphery (Lewis et al., 1931; Deneau et al., 1953; Smith et al., 1968). From early investigations, subthreshold, non-algogenic doses of bradykinin were shown to lower pain thresholds in man (Sicuteri, 1965) and dog (Lim and Guzman, 1968), and synergism between chemical substances was also demonstrated for histamine (Lewis, 1942; Emmelin and Feldberg, 1947), acetylcholine (Skouby, 1953), bradykinin (Sonia and Khaitin, 1967) and 5-HT (Sicuteri et al., 1965). The discovery of the hyperalgesic effects of the prostaglandins by Ferreira (1972) was followed by numerous investigations which resulted in the E-series prostaglandins being

considered as the most important endogenous mediators of hyperalgesia (Moncada et al., 1975).

Many studies have demonstrated the excitatory effects of algogenic substances on nociceptive sensory receptors. Receptors with A-delta and C fibre afferents have been examined in a variety of tissues including the skin (Douglas & Ritchie, 1960; Fjallbrant & Iggo, 1961; Beck & Handwerker, 1974; Chahl & Iggo, 1977), viscera (Douglas & Ritchie, 1957; Armett & Ritchie, 1961; Niijima, 1971; Kumazawa & Mizumura, 1980a,b; Delpierre et al., 1981; Cottrell & Iggo, 1984), muscles (Mense & Schmidt, 1974, Franz & Mense, 1975; Fock & Mense, 1976; Hiss & Mense, 1976; Kumazawa & Mizumura, 1976; Mense, 1977; Foreman et al., 1979; Mense, 1982), and joints (Schaible & Schmidt, 1983a,b; Kanaka et al., 1985). These studies provide valuable information concerning the effects of algogenic chemicals on the response characteristics of sensory receptors. However, it is not possible using this approach to study in detail the ionic mechanisms responsible for the generation of action potentials within sensory endings.

Excitatory responses to applications of various algogenic substances have been demonstrated for sensory neurone somata in the nodose ganglion (Sampson & Jaffe, 1974; Higashi, 1977; Higashi et al., 1982). It has also been shown that transport of receptors for various substances from cell bodies to peripheral nerve terminals occurs in the vagus nerve (Young et al., 1980). These findings suggest that the somata, axons and sensory terminals of primary afferent neurones may possess the same receptors for algogenic substances. Relying on the assumption that these receptors function in a similar manner and are associated with the same ionic channels in each location, changes in membrane properties produced

by various algogens have been investigated in electrophysiological studies on the axons and cell bodies of sensory neurones.

1.9.1 Bradykinin

The nonapeptide bradykinin is formed in blood and tissue from the breakdown of high molecular weight precursors, the kininogens, by the proteolytic actions of the plasma kallikreins (Rocha e Silva, 1964). Under normal circumstances, blood and tissue levels of bradykinin are very low, but during inflammation the processes of formation are highly active and peripheral levels are markedly increased (see Lewis, 1970; Garcia-Leme, 1978). Tissue damage induces activation of Hageman factor resulting in the conversion of prekallikreins to their active form and the subsequent production of bradykinin (see fig. 1.1).

Bradykinin triggers a number of specific pro-inflammatory responses in different tissue types including fluid secretion by epithelia (Manning et al., 1982), contraction of venous smooth muscle leading to increased capillary permeability (Regoli & Barabe, 1980), release of histamine and eicosanoids from various cells involved in the inflammatory process (Regoli & Barabe, 1980; Marceau et al., 1983), and stimulation of cell growth (Rozengurt, 1986).

1.9.1.1 Bradykinin and pain

Bradykinin is generally considered to be the most potent endogenously ocurring algogenic substance. Original investigations demonstrated that when applied to a blister base in man bradykinin produced painful

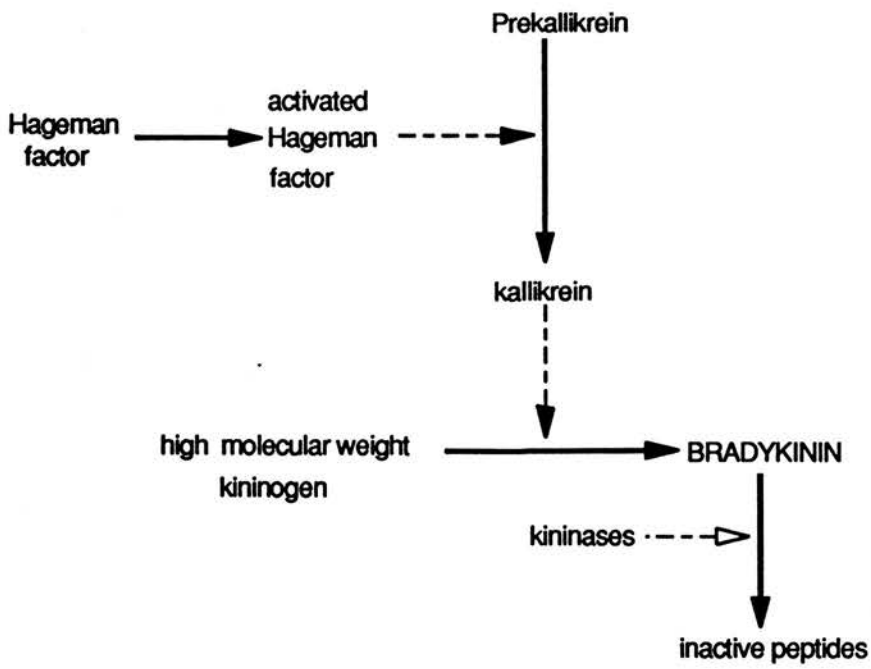

Fig 1.1 Schematic diagram showing the generation and breakdown of bradykinin. Solid arrows indicate transformations and dashed arrows indicate substances activating a transformation.

sensation (Keele & Armstrong, 1964), and when injected intra-arterially in dogs induced strong pseudoaffective responses indicative of pain (Guzman et al., 1962). In subsequent studies bradykinin injected intradermally (Lim et al. 1967; Ferreira, 1972) intra-arterially (Burch and Pasquale, 1962; Coffman, 1966) or intra-abdominally (Cormia & Dougherty, 1960; Lim et al., 1967) produced pain in humans, or when injected intra-arterially (Hashimoto et al., 1964; Ferreira et al., 1973) or intra-articularly (Melmon et al., 1967; Moncada et al., 1975) in dogs, induced pseudoaffective responses. These potent algesic effects of bradykinin are not, however, found by all workers. In man, a sensation of warmth rather than pain may be experienced (Fox et al., 1961), and bradykinin-induced pain is found to be dependent on the presence of other inflammatoy mediators such as 5-HT or prostaglandins (Sicuteri et al., 1965; Ferreira et al., 1973; Moncada et al., 1975). Bradykinin is in fact one of the most potent endogenous agents in its ability to release prostaglandins (Juan et al., 1984) via phospholipase A2 (Juan, 1977). Furthermore, in the blister base preparation, pain induced by bradykinin is prone to tachyphylaxis (Keele and Armstrong, 1964), whereas no tachyphylaxis occurs in the hand vein following exposure to 5-HT (Sicuteri et al., 1965) or when bradykinin is injected intra-arterially in dogs (Guzman et al., 1962).

These observations, together with the low levels of kinins found in inflammatory exudates and the lack of correlation between pain severity and kinin concentrations (Melmon et al., 1967) suggest that bradykinin is not the sole mediator responsible for the pain associated with inflammation, although it may well play a role.

1.9.1.2 Bradykinin and nociceptive sensory nerves

It is clear from recordings of afferent activity from cutaneous sensory receptors that bradykinin, nonselectively excites both nociceptors and low-threshold slowly adapting mechanoreceptors when injected intra-arterially (Fjallbrant & Iggo, 1961; Beck & Handwerker, 1974) Variability in the reported potency of bradykinin on cutaneous C fibre afferent units has also complicated analysis of its actions (Fjallbrant & Iggo, 1961; Beck & Handwerker, 1974; Chahl & Iggo, 1977). Bradykinin excites nociceptive sensory receptors present in visceral tissues (Kumazawa & Mizumura, 1976; Haupt et al., 1983), skeletal muscle (Mense & Schmidt, 1974; Franz & Mense, 1975; Kumazawa & Mizumura, 1976; Kumazawa & Mizumura, 1977a) and joints (Kanaka et al. 1985). In all of these tissues, tachyphlaxis of responses to bradykinin has been reported. In contrast to the situation described in the skin, in muscle and joint tissues bradykinin is reported to excite A-delta and C fibre afferent units selectively without affecting those with fast conducting axons (Mense, 1977; Kanaka et al., 1985). Bradykinin-induced sensitization of A-delta and C fibre muscle nociceptors to mechanical stimulation has also been demonstrated (Mense and Meyer, 1987).

The excitatory actions of bradykinin on nociceptors in all tissues are found to be enhanced in the presence of other chemical mediators such as 5-HT (Mense, 1981) and prostaglandins (Chahl & Iggo, 1977; Mense, 1981). Furthermore, bradykinin-induced activation of muscle (Mense, 1982) or testicular (Kumazawa & Mizumura, 1980b) nociceptors is reduced following administration of aspirin or indomethacin respectively. These results provide evidence to suggest that the excitatory effects of bradykinin

are at least partially dependent on the actions of other substances on nociceptive sensory receptors.

Clues to the mechanisms behind the excitatory effects of bradykinin on nociceptive nerve terminals are supplied by studies examining the membrane permeabilty changes evoked by bradykinin in the cell bodies of sensory neurones (Baccaglini & Hogan, 1983; Higashi et al., 1982; Lindsay & Rang, 1987; Weinreich, 1986). In dissociated dorsal root ganglion cells from adult rats, bradykinin produces a long-lasting (1-4 minutes) inward current associated with increased conductance, and a second inward current, at depolarized membrane potentials associated with inhibition of a voltage sensitive K^+ current (Rang & Lindsay, 1987). A similar effect of bradykinin is seen in a subpopulation of C-type nodose ganglion cells in the rabbit where inhibition of a slow spike after-hyperpolarization is produced via blockade of a Ca^{2+}-dependent K^+ conductance (Weinreich 1986).

Bradykinin is known to produce effects in many tissues via the activation of second messenger systems (Miller, 1987), and recently, bradykinin has been shown to increase production of inositol 1,4,5-triphosphate and diacylglycerol in neonatal rat sensory neurones in culture (Burgess et al., 1987). The use of phorbol esters to mimic the effects of diacylglycerol depolarizes and increases membrane permeability of sensory neurones <u>in vitro</u> (Lindsay & Rang, 1987; Burgess et al. 1989). Furthermore, the effects of bradykinin or phorbol dibutyrate are blocked by the protein kinase C inhibitor staurosporine. Densensitization to phorbol dibutyrate abolishes the response to bradykinin, suggesting a common mechanism of action involving protein kinase C (Bettaney et al., 1988b). Similar depolarizing effects of

bradykinin observed in mouse neuroblastoma x rat glioma cell hybrids are produced via the inhibition of a voltage-dependent K^+ current (Brown & Higashida, 1988a). This response also appears to be mediated via activation of protein kinase C, as it is closely reproduced by application of phorbol esters (Brown & Higashida, 1988b). Finally, using the isolated neonatal spinal cord and tail, Dray et al. (1988) have demonstrated that bradykinin and phorbol esters stimulate capsaicin sensitive C fibres by a mechanism involving protein kinase C, suggesting that the effects observed on sensory neurone somata also operate at the peripheral terminals of these cells.

1.9.1.3 Receptors for bradykinin

The varied actions of bradykinin in different tissues are mediated through at least two types of bradykinin receptor which have been named B1 and B2 receptors (Regoli & Barabe, 1980). This classification was based on order of agonist potency and on the effects of specific B1 receptor antagonists (Regoli & Barabe, 1980). Original studies were carried out using various smooth muscle preparations where B1 receptors are present in rabbit aorta (Regoli et al., 1977) and rabbit mesenteric veins (Regoli et al., 1978), and B2 receptors are present in rabbit jugular vein (Regoli et al., 1978), rat uterus (Barabe et al., 1977), cat ileum (Barabe et al., 1977) and several other tissues. The B1 receptor is particularly interesting as it has been found to be synthesized de novo in the mesenteric vein, aorta and urinary bladder during in vitro incubation and during inflammation (Regoli et al., 1978; Marceau et al., 1980). The development of B2 receptor antagonists by

Vavrek & Stewart (1985) has been followed by the description of a complex series of B2 receptor subtypes in a range of tissues (Rifo et al., 1987; Steranka et al., 1988).

Evidence is accumulating that the effects of bradykinn on nociceptive sensory nerves are dependent on activation of a B2 receptor. Pain evoked by application of bradykinin to the blister in man has recently been shown to be blocked by a B2 receptor antagonist, while a selective B1 receptor agonist had no algesic effect (Whalley et al., 1987). Bradykinin-induced excitation of canine, testicular polymodal nociceptors in vitro has recently been found to be B2 receptor mediated (Mizumura et al., 1990).

In recent studies demonstration of B2 receptor mediated effects on isolated cells has also been achieved. In C-type rabbit nodose ganglion cells bradykinin-induced inhibition of the slow after hyperpolarization has been demonstrated to be B2 receptor mediated (Rang & Heyman, 1990), and inhibition of the voltage-dependent K^+ conductance in mouse neuroblastoma x neuroma hybrid cells is also mediated through a B2 receptor (Brown & Higashida, 1988a).

1.9.2. 5-Hydroxytryptamine

5-Hydroxtryptamine (5-HT, serotonin) is synthesized in a variety of tissues, including enterochromaffin cells, neurones and rat mast cells, via the hydroxylation and deamination of tryptophan. Blood platelets, although not possessing the capacity for synthesis, actively store 5-HT. Although circulating levels of free 5-HT in plasma are low (Garattini & Valzelli, 1965; Franzen & Eysell, 1969), platelets may release their

contents during immune activation or as a result of tissue damage. 5-HT may also be released from mast cells by agents such as substance P (Johnson & Erdos, 1973). Released 5-HT is either taken up again by platelets, or else metabolized by the enzyme monoamine oxidase.

5-HT is highly vasoactive and causes smooth muscle contraction and increased capillary permeabilty (Levy, 1974; Douglas, 1975). Since 5-HT is not found in mast cells and basophils in man, it has not generally been considered to be an important mediator of inflammatory reactions in humans. However, recent investigations have revealed that platelets play an active part in the progression of a number of inflammatory diseases (see Page, 1988), suggesting a role for 5-HT in man.

1.9.2.1 5-HT and pain

The algogenic properties of 5-HT were established by Keele and Armstrong (1964) who demonstrated its ability to induce pain when applied to a blister base in humans. When injected intra-arterially, 5-HT was also shown to induce psudoaffective response in dogs (Guzman et al., 1962). Sicuteri (1965) demonstrated the sensitizing properties of 5-HT on the pain-inducing effects of bradykinin in man, and later proposed a role for the amine in the pain of thrombo-embolic disorders and migraine (see Sicuteri, 1968). Sensitization to bradykinin-induced pseudoaffective response in animals has also been observed (Ferreira, 1973; Nakano & Tiara, 1976).

1.9.2.2 5-HT and nociceptive sensory nerves

Excitation of C fibre visceral afferents following the injection of 5-HT into the circulatory supply of the visceral organs was shown by Douglas & Ritchie (1957a). In further experiments, Douglas & Ritchie (1957b) found that 5-HT was a very potent excitant of cutaneous receptors. This finding was supported in single unit studies by Fjallbrant & Iggo (1961), who demonstrated that both high threshold receptors with non-myelinated afferents and low-threshold slowly adapting mechnoreceptors with myelinated axons were excited by 5-HT. This non-specificity of action on cutaneous receptors was also demonstrated by Beck & Handwerker (1974).

In contrast to its non-selective effects in skin, 5-HT excites C and A-delta receptors from cat muscle with no effect on those receptors with faster conducting afferents (Mense & Schmidt, 1974; Fock & Mense, 1976; Mense, 1977;). In cat muscle, 5-HT has also been shown to sensitize C fibre receptors to the excitatory effects of bradykinin (Mense, 1981). In experiments using sections of rabbit vagus nerve <u>in vitro</u>, Neto (1978) demonstrated that 5-HT induced a depolarization of non-myelinated C fibres. Furthermore, the cell bodies of vagal primary afferents in the nodose ganglion are excited and produce action potentials in response to application of 5-HT (Sampson & Jaffe, 1974; De Groat & Simonds, 1976). A direct depolarizing action on these neurones has been demonstrated by several groups of workers (Higashi, 1977; 1980; Simmonds & De Groat, 1980; Wallis et al., 1982). The majority of C type nodose ganglion neurones responded to 5-HT with a rapid depolarization, due mainly to simultaneous increases in Na^+ and K^+ conductances, followed by a

hyperpolarization brought about by an increase of K^+ conductance triggered by a voltage-dependent influx of Ca^{2+} (Higashi & Nishi, 1982). This after-hyperpolarization was occasionally followed by a long-lasting depolarization associated with a small, but sustained, increase in Na^+ conductance. Appearance of the initial depolarization is dependent on the method of application of 5-HT, and is susceptible to tachyphylaxis (Higashi, 1977; Wallis & Dun, 1988). Similar responses to application of 5-HT have been demonstrated in bull frog type C dorsal root ganglion cells. (Morita & Katayama, 1986). In these cells, a rapid depolarization was due to an increase in membrane conductance to Na^+ and K^+, a hyperpolarization was caused by activation of a Ca^{2+}-dependent K^+ conductance, and a depolarization of delayed onset was due to the inactivation of a K^+ conductance.

5-HT has recently been shown to increase the excitability of a sub-population of C-type nodose ganglion cells from the rabbit via the blockade of a Ca^{2+}-dependent K^+ conductance (Christian et al., 1989).

1.9.2.3 Receptors for 5-HT

5-HT has a vast range of reported actions, including effects on vertebrate smooth muscle, neuronal cell bodies, dendrites, nerve fibres, terminals, blood platelets and glandular function. In the 1950s, Gaddum & Piccarelli (1957) defined two types of 5-HT receptor in smooth muscle, the M and the D receptors. Responses mediated by the M receptor could be blocked by morphine, while those mediated by D receptor could be blocked by dibenzyline (phenoxybenzamine) and by LSD (d-lysergic acid diethylamide). It has since been found that the functional antagonism

produced by morphine and phenoxybenzamine probably do not occur at the level of the 5-HT receptor, while that of LSD does (Paton, 1957; Kosterlitz & Robinson, 1958).

More recently, receptor binding studies using selective agonists and antagonists have defined three major types of serotonin receptors (see Bradley et al., 1986; Kilpatrick et al., 1987; Peroutka, 1988). According to classification based on functional criteria (Bradley et al., 1986), the three receptor types have been designated 5-HT_1-like, 5-HT_2 and 5-HT_3. The 5-HT_1 receptor has been further subdivided into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} mainly on the basis of data from binding studies, although some functional correlates have also been proposed (see Peroutka, 1988).

The excitatory action of 5-HT on primary afferent neurones has invariably been found to be mediated through the 5-HT_3 receptor subtype as defined by Bradley et al. (1986). Thus, 5-HT-induced excitation or depolarization of sensory neurones can be mimicked by the 5-HT_3 receptor agonist 2-methyl 5-HT, are potently antagonized by selective 5-HT_3 receptor antagonists such as MDL 72222 or ICS 205-930, and is not inhibited by ketanserin which selectively blocks 5-HT_2 receptors or methysergide and methiothepin which block both 5-HT_1 and 5-HT_2 receptors.

In the blister base preparation, 5-HT-induced pain is mimicked by the 5-HT_3 receptor agonists phenyl biguanide (Fastier et al., 1959) or 2-methyl 5-HT, is potently and competitively blocked by ICS 205-930 and unaffected by methysergide (Donatsch et al., 1984b; Richardson et al., 1985).

The rapid depolarization induced by 5-HT in rabbit isolated nodose

ganglion cells is mimicked by phenyl biguanide (Wallis et al., 1982), and is competitively blocked by MDL 72222 or ICS 205-930 (Azami et al., 1985; Round and Wallis, 1985; Round and Wallis, 1986; Christian et al., 1989). This effect of 5-HT cannot be blocked by methysergide (Higashi and Nishi, 1982). Similar depolariztion responses to 5-HT have been reported for the rabbit or rat isolated vagus nerve where the response is mimicked by phenylbiguanide (Ireland and Tyers, 1987), and can be blocked by 5-HT_3 antagonists (Donatsch et al., 1984a; Richardson et al., 1985).

Several reports provide evidence to suggest that 5-HT receptors other than 5-HT_3 are responsible for some of its actions on sensory neurones. 5-HT-induced hyperpolarization of the rabbit isolated vagus nerve occurs as a secondary event to the initial depolarization and as such is blocked by 5-HT_3 antagonists (Ireland, 1987). However, the existence of independent mechanisms mediating hyperpolarization was demonstrated in bullfrog type C dorsal root ganglion cells, where methysergide reversibly blocked the hyperpolarization but had no significant effect on initial depolarization (Morita and Katayama, 1986). The delayed onset depolarization induced by 5-HT in bullfrog dorsal root ganglion cells was blocked by methysergide in type A neurones (Morita and Katayama, 1986). Finally, in rabbit nodose ganglion cells 5-HT-induced inhibition of a Ca^{2+}-depedent K^+ conductance is resistant to ICS 205-930 but is antagonised by methysergide (Christian et al., 1989).

1.9.3. Prostanoids

The prostanoids are a group of polyunsaturated, 20 carbon chain fatty acids. They are synthesized via the metabolism of polyunsaturated fatty acid precursors (Van Dorp et al.1964; Bergstom et al., 1964) by the enzyme fatty acid cyclooxygenase (Hamberg et al., 1974). Cyclooxygenase is present in most animal cells (Christ & Van Dorp, 1972) where it has been shown to be associated with the microsomal cell fraction (Nugteren et al., 1966; Samuelsson 1967; Miyamoto et al., 1974; Rollins & Smith, 1980). The preferred substrate for this enzyme is arachidonic acid, but dihomo-γ-linoleic acid and eicosapentanoic acid can also be converted to form prostanoid products (Van Dorp, 1967).

Free arachadonic acid can be metabolized to form the unstable prostaglandin endoperoxides which are in turn enzymatically converted to form PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2 (Moncada et al., 1976), or TXA_2 (Hamberg et al., 1975) (fig 1.2). The metabolite formed varies from cell to cell as illustrated by the observations that in blood platelets arachidonic acid is converted to TXA_2, whereas vascular enothelium produces mainly PGI_2 (see Samuelsson et al., 1978)

Cyclooxygenase activity is increased by the application of mechanical or chemical stimuli (Ferreirra & Vane, 1967) or by immunological challenge (Piper & Vane, 1969). Willis (1978) was the first to discover prostanoid activity in inflammatory exudate, and subsequently PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2 and TXA_2 (the latter two being measured as their stable metabolites 6-keto-$PGF_{1\alpha}$ and TXB_2 respectively) were detected in inflamed tissues both from animals and man (see Higgs et al., 1984).

During inflammation, prostanoids are likely to be produced by a number

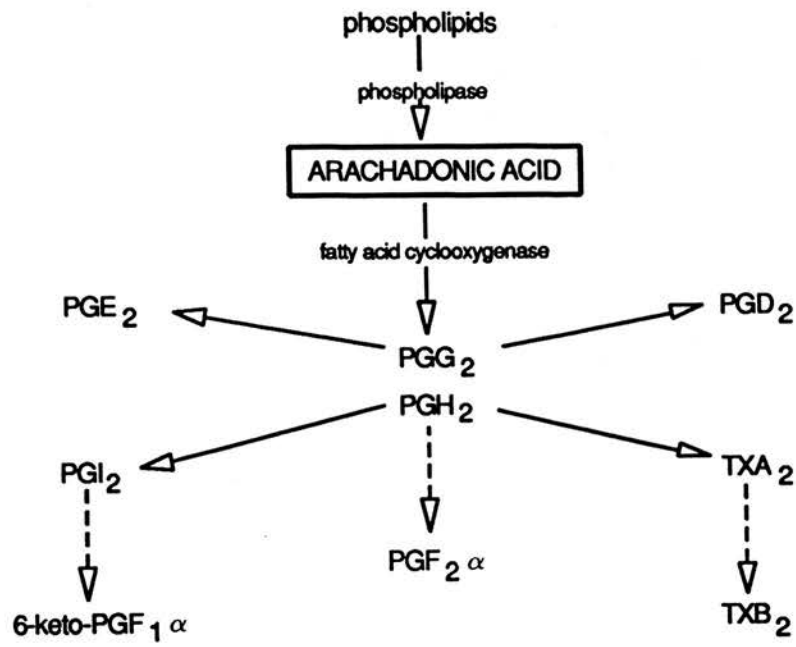

Fig. 1.2 Schematic diagram showing the major prostanoid products of arachidonic acid metabolism. Enzymically controlled reactions are shown as solid line arrows.

of different cell types including platelets (Willis, 1978), mast cells (Becker & Henson, 1973; Roberts et al., 1978), neutrophils (Goldstein et al., 1977; Zurier, 1976), mononuclear phagocytes (Davies & Allison, 1976) and macrophages (Myatt et al., 1975; Gordon et al. 1976; Humes et al., 1977). Prostanoids are also produced by inflamed tissues ex vivo such as cultured human synovium, which produces PGE_2 (Robinson et al., 1975), PGI_2 and TXA_2 (Salmon et al., 1983).

The four cardinal signs of inflammation are all induced by metabolites of arachidonic acid. This is due in part to their ability to dilate blood vessels and increase vascular permeability (Lewis et al. 1977). PGE^1 and PGI_2 produce local vasodilatation and increase vascular permeability when injected into the skin of animals (Horton, 1963; Crunkhorn & Willis, 1971a; Kaley & Weiner, 1971 ; Peck & Williams, 1978) and man (Crunkhorn & Wilis, 1971; Kuehl, 1977). Arachidonic acid derivatives are poor at eliciting plasma exudation when injected alone into guinea-pig (Horton 1963, Williams & Morley 1973; Williams, 1976) or rabbit (Williams 1976) skin, but they do potentiate plasma exudation produced by other mediators (Moncada et al., 1973; Williams & Morely, 1973; Williams, 1976). PGE_2, $PGF_{2\alpha}$ and PGI_2 also potentiate irritant-induced oedema in animals (Higgs et al, 1978; Murota et al., 1979; Williams & Morely, 1973; Williams & Peck 1977).

1.9.3.1 Prostanoids and pain

Horton (1963) found that PGE_1 did not produce pain when applied to a blister base, and similarly Crunkhorn & Willis (1971b) demonstrated that intradermal injection of microgram doses of the E-series prostanoids

produced local oedema and erythema but no discomfort or pain. However, the oedematous area caused by the injection of PGE_1 was shown by Solomom et al. (1968) and Juhlin & Michaelsson (1969) to be hypersensitive to touch. Larger doses of E-type prostanoids can cause pain when infused into the human arm vein (Collier et al., 1972), or headache when injected intravenously (Bergstrom et al., 1959). Intra-articular injection of PGE_1, PGE_2 or, to a lesser extent, $PGF_{2\alpha}$ induced an incapacitation in the dog knee joint (Rosenthal et al. 1972), and when injected intra-peritoneally in mice, PGE_1 and PGE_2 induced a writhing response (Collier & Schneider, 1972).

A clear dissociation between the pain-producing effects of PGE_1 when administered at high doses and the hyperalgesic effects of low doses was demonstrated in a series of experiments conducted by Ferreira (1972). Bolus intradermal injections of a high dose of PGE_1 in man produced an intense and prolonged pain. This was in contrast to the intense, short lived pain induced by intradermal acetylcholine or bradykinin. Of particular interest was the observation that intradermal infusions of lower doses of bradykinin produced only mild pain, while the concurrent infusion of low dose PGE_1 with bradykinin induced strong pain.

The potentiating effects of PGE_1 and PGE_2 have been reported in a number of different models including psuedoaffective response induced by injection of bradykinin into the spleen of the dog or cat (Ferreira, 1972, Tyers & Haywood, 1979), and rat paw hyperalgesia (Willis & Cornelson, 1972; Ferreira et al., 1978; Tyers & Haywood, 1979). Comparison of PGE_2- and PGI_2-induced effects has shown that the two prostanoids produce similar hyperalgesic effects in the dog knee joint and in the rat paw (Ferreira et al., 1978). The reflex fall in blood

pressure produced by intra-arterial injection of bradykinin in the rabbit ear was used by Juan (1979) to examine the potentiating effects of PGE_1, PGE_2 and PGI_2. In this model PGE_1 and PGI_2 were equipotent, while much larger doses of PGE_2 were required to produce an effect.

In a series of experiments in which nociceptive thresholds in rats were measured, Ferreira and his colleagues (Ferreira & Nakamura, 1979a; Ferreira & Lorenzetti, 1981) attempted to analyse the biochemical events causing prostanoid-induced hyperalgesia. Hyperalgesia was induced by subplantar injections of PGE_2, PGI_2, Ca^{2+}, or dibutyryl cAMP. These hyperalgesic effects were potentiated in the presence of phosphodiesterase inhibitors and reversal of PGE_2-induced hyperalgesia was produced by Ca^{2+} channel blockers. These results were interpreted to to support the idea that prostanoids induce hyperalgesia via a $cAMP/Ca^{2+}$-dependent mechanism (Ferrreira & Nakamura, 1979).

Evidence supporting a role for the prostanoids in inflammatory pain was provided by Vane and his colleagues (Vane, 1971; Ferreira et al., 1971) and Smith & Willis (1971) who demonstrated in several systems <u>in vitro</u> that non-steroidal anti-inflammatory drugs (NSAIDS) inhibit arachidonic acid metabolism and thus prostanoid production. By reducing levels of endogenous prostanoids, NSAIDS are effective anlgesics only in the pain associated with inflammation where prostanoid levels are elevated.

1.9.3.2 Prostanoids and nociceptive sensory nerves

The potentiating effects of prostanoids on the reponses of nociceptive sensory receptors to bradykinin has been demonstrated in skin for PGE_1

and PGE_2 (Handwerker, 1976; Chahl & Iggo, 1977), in cat muscle for PGE_2 (Mense, 1981) in canine testicular tissue for PGE_2 and PGI_2 (Kumazawa et al., 1987) and in cat knee joint for PGE_2 (Schaible & Schmidt, 1988b).

PGE_1- and PGE_2-induced sensitization of cutaneous sensory receptors to mechanical stimuli has been shown for C polymodal nociceptors (Martin et al., 1987) and A-delta mechanoreceptors (Petromichelakis & Rood, 1982; Martin et al., 1987). PGE_2-induced sensitization to movement of A-delta mechanoreceptors, and to a lesser extent C fibre mechanoreceptors, has also been shown in the cat knee joint (Schaible & Schmidt, 1988). In addition to sensitization, excitatory effects of the prostanoids have been reported for PGE_1 in rat skin (Chahl and Iggo, 1977) and for PGE_2 in the cat knee joint (Schaible & Schmidt, 1988). In these studies, prostanoid-induced excitation was generally found to be longer lasting than that produced by other excitants such as bradykinin.

Potentiating effects of prostanoids on responses of sensory receptors and facilitatory effects on primary afferent neurotransmision have been demonstrated using the neonatal rat <u>in vitro</u> spinal cord-tail preparation (Yanagisawa et al., 1986). PGE_1 or PGE_2 applied to the tail markedly potentiated capsaicin-induced reflex ventral root depolarization. This effect was not obtained with PGD_2 or $PGF_{2\alpha}$. In addition, application of PGE_1 or PGE_2 to the spinal cord increased the amplitude and duration of capsaicin-induced dorsal root depolarization.

Electrophysiological studies on rat sensory neurones in culture have shown that application of PGE_2 causes these cells to produce action potentials (Baccaglini & Hogan, 1983). In rabbit C-type nodose ganglion neurones PGE_1, PGE_2, PGD_2, but not $PGF_{2\alpha}$ inhibit a slow after-

hyperpolarization via blockade of a Ca^{2+}-dependent K^+ conductance (Fowler et al., 1985a; Fowler et al., 1985b; Weinreich & Wonderlin, 1987). This effect of the prostanoids on C-type nodose ganglion cells was mimicked by forskolin suggesting that inhibition of the Ca^{2+}-dependent K^+ conductance is mediated via cAMP (Weinreich & Wonderlin, 1987).

1.9.3.3 Prostanoid receptors

A working classification of receptors has recently been developed based mainly on functional criteria (Kennedy et al., 1982; Coleman et al., 1984; Jones et al., 1984; Coleman et al., 1985). Within the classification there are receptors which are specific for each of the five naturally occuring prostanoids: PGD_2; PGE_2; $PGF_{2\alpha}$, PGI_2 and TXA_2. Coleman et al. (1984) have developed a system of nonmenclature in which each of the five basic prostanoid receptors are designated DP, EP, FP, IP and TP where the letter 'P' stands for prostanoid and the preceding letters indicate the natural prostanoid to which that particular receptor is most sensitive.

Despite the proposed existence of specific receptors for each prostanoid it is important to note that a high degree of cross reactivity exits beween receptor types. $PGF_{2\alpha}$, for example, causes contraction of guinea-pig trachael smooth muscle not through an action at FP receptors, but through interaction with EP- and TP-receptors (Coleman & Kennedy, 1985).

Further subdivision of the five basic prostanoid receptors has also been suggested based on various lines of evidence. EP receptors are

comprized of at least three subtypes (Coleman and Kennedy, 1985; coleman et al., 1987), while DP- and TP-receptors may each be comprized of two subtypes (Jones et al., 1984). The existence of such receptor subtypes is indicated by subscript numbers such that EP-receptors are termed EP_1-, EP_2 or EP_3-receptors.

As outlined above, the receptor classification was developed through studies on the actions of prostanoids on smooth muscle and blood platelets (Coleman et al., 1987). On smooth muscle prostanoids may cause either contraction via EP_1-, EP_3-, FP- or TP-receptors, or relaxation via DP-, EP_2- or IP-receptors. On blood platelets they may cause aggregation via TP-receptors or disaggregation DP- or IP-receptors. Prostanoid effects on other tissues include inhibition of gastric acid secretion via EP-receptors and luteolysis in a range of animal species via FP-receptors (Coleman et al., 1985).

Although the link between prostanoids and hyperalgesia is well established, little work has been carried out to investigate the effects of more selective prostanoid analogues. The high potency of PGE_1 and PGE2 supports the involvement of EP-receptors, while the reports of high potency of PGI_2 suggest that IP-receptors may also be involved.

1.10 Adjuvant arthritis as a model for the study of the pain associated with chronic inflammation

1.10.1 Pathology and aetiology

The first report on the appearance of a polyarthritis in rats injected with spleen extracts emulsified in Freunds complete adjuvant (heat killed*Mycobacterium*) was made by Stoerk et al. (1954). Pearson (1956)

extended these observations, and demonstrated that Freunds complete adjuvant alone was capable of inducing polyarthritis. Adjuvant polyarthritis has been reviewed by several authors in the past (Swingle, 1974; Rosenthale, 1974; Billingham & Davies, 1979; Rainsford, 1982; Billingham, 1983).

As originally described by Pearson (1956), adjuvant-induced polyarthritis is a whole animal disease in which inflammation of multiple joints develops over a period of about ten days following injection of adjuvant into the base of the tail. The most severe inflammation occurs in the distal joints of the hindlimbs where there is a progressive destruction of bone, damage to tendons, and a loss of cartilage following prolonged cellular infiltration of the synovium. Additional observations on the disease include an increase in the size of the lymph nodes, a marked impairment of liver metabolism and a series of serum and tissue chemical changes (see Rainsford, 1982).

The inhibitory effects of steroids on adjuvant arthritis were first described by Pearson & Wood (1959), and the model was developed as a screening test for anti-arthritic drugs by Newbold (1963). The shortcomings of studying acute models of inflammation in the testing of drugs to treat a chronic condition was pointed out at this time (Newbold, 1963).

When suspended in vegetable or mineral oils, many species of dead Mycobacterium are capable of inducing adjuvant arthritis in the rat (see Billingham, 1983). Induction of polyarthritis has also been achieved using other species of bacteria (Flax & Waxman, 1963; Paronetto, 1970), water soluble peptidoglycans (Kohashi et al, 1976; 1977) and muramyl dipeptide derived from Mycobacterium (Kohashi et al., 1980).

Many different factors are important for the successful and consistent induction of adjuvant polyarthritis, including the concentration of Mycobacterium and its degree of dispersion (Ward & Jones, 1962; Newbold, 1963), the site of adjuvant injection (Winder et al., 1969; Perper et al., 1971; Koga et al., 1976) and the strain of rat used (Freeman & West, 1972; Eisen et al., 1973; Zideck & Perlik, 1971).

The development of adjuvant arthritis was originally considered to be dependent on the involvement of immune mechanisms. Evidence from a number of workers favoured a delayed-type T-cell mediated hypersensitivity response to a disseminated antigen (Pearson & Wood, 1959; Waksman et al., 1960; Flax & Waxman, 1963; Waxman & Wennerstein, 1963; Gery & Waksman, 1967). However, immunity to myobacterial components does not appear to be essential for disease expression as a non-immunogenic muramyl dipeptide can induce adjuvant polyarthritis (Kohashi et al., 1980). Furthermore, another non-immunogenic, low molecular weight material, an interferon inducer (CP 20961) is capable of initiating an arthritis similar to classical polyarthritis (Chang et al., 1980). Immunity to the inducing agent, either humoral or cell mediated, has been shown to have an exacerbatory influence rather than an initiating role in the development of adjuvant arthritis (MacKenzie et al., 1978; Smith et al., 1982). Dissemination of the injected material to various sites, including synovial membranes (Vernon-Roberts et al., 1976), would determine the site of activity with T-cell antibody, but this appears to be a separate event to that causing the degradative bone changes seen in adjuvant arthritis (see Billingham, 1983).

1.10.2 Studies in the pain associated with chronic inflammation

A large number of researchers have found that the development of adjuvant arthritis is associated with the a measurable hyperalgesia. A wide variety of assessment techniques have been used, including the Randall-Selitto test (Kayser & Guilbaud, 1981; Kayser & Guilbaud, 1983; Hara et al. 1984; Butler et al., 1985; Calvino & LeBars, 1986; Calvino et al., 1987), tail-flick test (Colpaert, 1979; Yonehara et al., 1983), hot-plate test (Hara et al., 1984), rotarod grip strength (Perrine & Takesue, 1968), squeezing with forceps (Hirose & Jyoyama, 1971), foot-bend procedure (Kuzuna & Kawai, 1975; Winter et al., 1979; Capetola et al., 1980; Calvino et al., 1987; Calvino & LeBars, 1988) and transcutaneous electrical stimulation (Oliveras et al., 1979; Okuyama & Aihara, 1984).

It has been suggested by Colpaert and his colleagues (see Colpaert, 1987) that the tests mentioned above represent meaurements of superimposed acute pain rather than chronic pain itself, and a number of investigations have been undertaken to validate adjuvant arthritis as a model of chronic pain.

Several approaches have been used in an attempt to relate behaviour to the presence of chronic pain. It has been shown that arthritic rats preferentially consume drinking water containing analgesic drugs (Colpaert et al., 1980), display disturbances in food intake (Colpaert et al, 1982; Colpaert & Van Den Hoogen, 1983; Calvino et al., 1987), show signs of irritability (Procacci et al., 1979; Reuler et al., 1980; Colpaert et al. 1982) and hyperventilate (Colpaert & Van Den Hoogen, 1983). Increases in breathing follow the same time course as impaired

weight control and paw swelling and are reduced by administration of opioids or aspirin-like drugs (Colpaert & Van Den Hoogen, 1983; Colpaert et al., 1987). Other behavioural parameters that have been used as representative measures of chronic pain are marked decreases in locomotion and increases in scratching behaviour (De Castro Costa et al., 1981; Calvino et al., 1987). Decreased locomotion is responsive to aspirin-like drugs (Larsen & Arnt, 1985), and increased scratching behaviour can be reduced by morphine or aspirin-like drugs (De Castro Costa et al., 1987).

1.10.3 Nociceptor characteristics in adjuvant polyarthritis

The first studies carried out to determine the changes occurring in nociceptive systems occuring as a result of adjuvant arthritis were directed towards the central nervous system. These showed that at various levels of the sensory pathway many neurones had an increased resposiveness to peripheral stimuli. Neurones from spinal dorsal horn (Menetrey & Besson , 1982), thalamus (Gautron & Guilbaud, 1982; Kayser & Guilbaud, 1984) and cortex (Lamour et al., 1983) which normally only respond to intense noxious stimuli have been shown to respond to mild mechanical stimuli in arthritic rats. Pressure stimuli in the ankle joint region of a normal rat induces responses in spinal dorsal horn, thalamic or cortical neurones which can be activated by gentle cutaneous mechanical stimuli. In contrast to the situation in normal rats light pressure stimuli on the ankle joint of an arthritic rat evokes vigorous and sustained discharge in neurones of the superficial dorsal horn, an area which contains large numbers of nociceptor-specific neurones

(Menetrey & Besson, 1982).

It has been suggested that the changes described above could be related to alterations in the processing of nociceptive information in the central nervous system occurring as a result of an elevated peripheral input (Menetrey et al. 1988). However, increased responsiveness to stimuli given in the periphery may be due to alterations in sensory receptor responsiveness. The possible involvement of peripheral nociceptive sensory elements in these effects has been investigated by Guilbaud and colleagues.

Guilbaud et al. (1985) examined the characteristics of nociceptors from the tibiotarsal (ankle) joints of rats with established adjuvant-induced polyarthritis. High-threshold mechanoreceptors with C fibre afferents were found to have lower mechanical thresholds in arthritic joints than those in normal joints. High levels of ongoing resting discharge in these receptors was also a characteristic of arthritic joints. Large numbers of receptive fields each corresponding to a single afferent unit could be found and activated by mechanical probing, this being in contrast to the situation in normal joints where receptive fields were sparse, and could be found only with great difficulty applying strong pressure stimuli. The sizes of individual receptive fields was no different in normal and arthritic joints.

Small movements of the ankle joint evoked a discharge from receptors in arthritic rats, whereas similar manipulations in normals were ineffective. Responsiveness of receptors to thermal stimuli in inflamed joints but not in normals provided evidence of further changes in receptor characteristics.

Histological studies of the nerve supply to the ankle revealed no

difference in number or size of axons between normal and arthritic rats. Any changes in sensitivity of the ankle joint to mechanical stimuli are not therefore due to change in the total number of afferent fibres (Guilbaud et al., 1985).

1.10.3.1 Inflammatory mediator involvement

From the evidence discussed in the previous introductory sections relating to the effects of inflammatory mediators on nociceptive sensory receptors, the next question to be answered is which of these mediators are responsible for the sensory changes seen in adjuvant-induced polyarthritis. In original studies it was demonstrated that intravenous injection of lysine acetylsalicylate (l-AS) reduced the responsiveness of ventro-basal thalamic neurones in arthritic rats (Guilbaud et al., 1982). A peripheral locus of action of this effect was later shown by Guilbaud & Iggo (1985). Following intravenous injection or topical application of l-AS in arthritic rats the responsiveness of articular high threshold receptors to controlled mechanical stimuli was reduced, as was any ongoing background discharge. It was concluded from these results that in arthritic joints the high level of excitability of these receptors could at least partly be attributed to the actions of arachidonic acid metabolites of the cyclooxygenase pathway.

1.11 An outline for the present investigation

It is clear that the pain and hyperalgesia of chronic inflammation results primarily from the actions of chemical mediators on sensory

receptors in the periphery. Recent investigations into chronic inflammatory pain have been dominated by the use of the adjuvant polyarthritic rat. The demonstration that administration of 1-AS reduces the enhanced sensitivity of articular sensory receptors seen in arthritic rats suggests that prostanoid products of arachadonic acid metabolism make a major contribution to the production of this phenomenon. Among the many putative inflammatory mediators it is not only the prostanoids that are capable of sensitizing or exciting sensory receptors with fine afferent fibres. Bradykinin and 5-HT are the most likely additional candidates for involvement in inflammatory pain.

With these points in mind the aim of the present investigation was to assess the actions of cetain prostanoids, bradykinin and 5-HT on articular sensory receptors in normal joints and in those affected by a localized adjuvant-induced arthritis. Furthermore, where possible the pharmacological receptors mediating the effects of endogenously produced and exogenously administered chemicals were to be characterized using selective agonist and antagonist compounds.

SECTION II

METHODS AND MATERIALS

SECTION II

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2.1 Induction of localized adjuvant-induced arthritis

Localized adjuvant arthritis was induced in male Wistar rats (200 - 250 g) by the local subdermal injection of Freunds complete adjuvant around the tibio-tarsal joint of the left hindlimb. Following induction of anaesthesia using ether or halothane, the left hindlimb was swabbed with alcohol, and three subdermal injections of a total volume of 0.15 ml Freunds complete adjuvant (1.0 $mgml^{-1}$ heat killed myobacterium tuberculosis suspended in paraffin oil, Sigma) were made into the tissues overlying the left ankle joint. Following recovery, the animals were housed in cages containing up to five rats. The development of inflammation was followed for up to 35 days during which period the animals were used for behavioural or neurophysiological experiments.

2.2 Assessment of the arthritic lesion

The development of the arthritic lesion was assessed using two groups of tests:

(a) Animals used in electrophysiological studies. In the case of these animals the general condition and activity of the rats was monitored on a daily basis by observing their behaviour in the cage up to the point at which they were used for an experiment. Routine measurements of joint

circumference were made using a measuring tape. Weight gain was monitored regularly along with visual assessment of secondary inflammatory lesion in the hind limbs, tail, snout and ears. Behaviour was assessed in relation to motility, scratching behaviour and stress during handling (see Colpaert, 1987).

(b) Animals used in behavioural studies. These studies were carried out to assess behavioural changes associated with the development of arthritis. Rats were subjected to the following series of observational and sensory tests involving the quantitative measurement of standing and walking weight load (Coderre & Wall, 1987), foot withdrawal to pressure (Randall & Selitto, 1957), foot withdrawal to heat, response to foot manipulation (Kuzuna & Kawai, 1975) and grading of foot placing reflexes (Coderre & Wall, 1987).

1. Standing weight load. Rats were placed in a 30 cm x 30 cm x 22 cm clear perspex box and observed for a period of five minutes. A mirror was set at an angle below the box to provide a clear view of the base of the rat's feet. The amount of weight the rat placed on each hindpaw was evaluated using the following scale:

0 - normal load bearing, paw placed firmly on the surface of the perspex floor;

1 - slightly reduced load bearing, paw completely in contact but without the toes being spread;

2 - moderately reduced load bearing, paw curled with only some parts of the foot lightly touching the

floor;

3 - severely reduced load bearing, paw lifted from surface;

2. Walking weight load. Rats were placed in the box, as described above, to assess any changes in gait or production of limp in the rats hindpaws caused by the induction of arthritis. Animals were scored as follows:

0 - normal gait;

1 - slight limp, over-flexion of the affected limb;

2 - moderate limp, paw briefly touches walking surface;

3 - severe limp, three-legged gait.

3. Foot withdrawal to pressure. Rats were hand-held, and the paws of the right and left hindlimbs were in turn placed under a metal bar which was lowered mechanically on to the paw exerting an increasing pressure (Ugo Basile, Comeria, VA, Italy), thus pushing the ventral aspect of the paw on to small raised blunt point. The apparatus was calibrated in grams of force. The force necessary to elicit a withdrawal response was used as the endpoint for the test. Thresholds were based on the average

of two trials. A cut-off point of 500 g was used in all tests.

4. **Foot withdrawal to heat.** Rats were hand-held, their right and left hindpaws being placed in turn over a small hole in the table surface beneath which was a small projector bulb which acted as a radiant heat source. The time taken for the rat to withdraw its paw was recorded. Latencies were based on the average of two trials.

5. **Paw withdrawal to mechanical indentation.** Rats were hand-held while resting on the bench and the withdrawal thresholds for both hindpaws were determined. Mechanical indentation stimuli around the ankle joint region were determined using calibrated von Frey hairs. Hairs were calibrated to exert forces of 0.2 - 90 mN at the first sign of bending.

6. **Foot manipulation.** Rats were hand-held, and their feet were gently flexed and extended over the normal working range of the ankle joint. Responses were classified as either noxious or non-noxious based on the presence or absence of vocalization or withdrawal when the paw was manipulated.

7. **Placing reflex.** Rats held by hand in the air were moved towards the edge of a table so that the dorsal surface of either the right or the left hindpaw just touched the side of the table. The response was classified as a placing reflex if the rat lifted its paw to place it on the top surface of the table. Scores were based on the number of clear reflexes observed out of five trials for each paw.

2.2.1 Statistical Assessment

Differences between groups were assessed using the Wilcoxon Mann-Whitney test and the null hypothesis rejected if $p < 0.05$.

2.3 Histological methods

Rats were anaesthetized with urethane (25% w/v, 0.6 ml/100g, i.p.), and a midline incision was made in the abdomen. A cannula was carefully inserted anterogradely into the abdominal aorta, and the abdominal vena cava was cut to allow the escape of blood and fixative from the hindquarters. Heparinized saline (500 Units/kg) was injected into the animal prior to perfusion with formal saline (NaCl, 0.9 g; 40% formaldehyde, 10 ml; distilled water, 90 ml) via the cannula in the abdominal aorta.

The hindlimbs were removed and post-fixed in formol saline overnight before being placed in a modification of Gooding and Stewart's (1932) decalcifying medium (formic acid 15 ml, 40% formaldehyde 5 ml, distilled water to 100 ml total volume) for two weeks. After decalcification, the tissues were trimmed around the tibio-tarsal joint and placed in 5% gelatin overnight at 37°C. Blocks were made in aqueous 10% gelatin which was allowed to set before being frozen in Arcton 12 (BOC) cooled with liquid nitrogen (-70°C). Sections of 20 um thickness were cut from each joint at 100 μm intervals using a cryostat (SLEE). The sections were mounted on glass slides, stained using haematoxylin and eosin, and sealed under glass coverslips using DPX. Slides were examined under a microscope, and the extent of joint pathology assessed. The presence of bone erosion, inflammatory cell infiltration and tissue fibrosis was determined for both injected and non-injected limbs.

2.4 Determination of tissue prostanoid levels

Rats were sacrificed by i.v. administration of barbiturate anaesthetic overdose (Euthetal) and the soft tissues surrounding the tibio-tarsal joint removed as quickly as possible and placed in 10ml ethanol. The tissues were stored in ethanol at -20°C for a period of 2 - 10 days at which time the now dehydrated tissues were blotted dry and weighed. The tissues were then homogenized with the ethanol in which they had been placed using a Fisons glass homogenizer. Each homogenate was centrifuged at 2500 x g for 10 minutes and the supernatant removed. The residue was washed with 5 ml ethanol and centrifuged again, the washings being removed and combined with the original supernatant. The ethanol was evaporated to dryness on a rotary evaporator at 50°C, and the dry extract dissolved in 10 ml distilled water. The acidity of the aqueous solution was lowered to pH4 with 0.1 M HCl, and the prostaglandins were extracted by shaking with ethyl acetate. The ethyl acetate was separated from the aqueous solution using a glass separator. This process was repeated with the remaining aqueous solution. The two ethyl acetate extracts were combined and evaporated to dryness on a rotary evaporator at 50°C. The dry residue was dissolved in 2 ml ethyl acetate, and stored at -20°C.

The amounts of PGE_2 and 6-keto-$PGF_{1\alpha}$ present in each extract were measured by radioimmunoassy, using antibodies whose cross reactivities have been reported previously (Dighe et al., 1975; Poyser, 1980; Poyser & Scott, 1980; Lytton & Poyser, 1982). The only significant cross-reactivities are $PGF_{1\alpha}$ (100%) with the $PGF_{2\alpha}$ antiserum, and PGE_1 (66%), PGA_2 (25.5%) and PGB_2 (11.8%) with the PGE_2 antiserum. The

inter-assay and intra-assay coefficients of variation for the three assays were all less than 10%. The limits of detection were 40 pg PGE_2 and 30 pg 6-keto-$F_{2\alpha}$ per assay tube for the respective assays.

2.4.1 Statistical assessment

Differences between groups were analysed using the Students T-test due to the small group numbers, and the null hypothesis rejected if $p < 0.05$.

2.5 Determination of plasma salicylate and paracetamol levels

Blood samples of 0.7 ml were taken at regular intervals using heparin coated syringes, and placed in labelled eppendorf tubes. The plasma and heamatocrit were separated by centifugation (Eppendorf 5414), the plasma layer being pipetted off and frozen at -20°C.

Total plasma salicylate was determined using a colorimetric kit (Sigma Diagnostics), and a colorimeter (Whatman DC 100). Determination was dependent on the reaction of salicylate with Ferric ions (Salicylate Colour Reagent, Catalogue No. 530-3) which produces a purple colour measured at 450 nM. Results were compared with those for a Standard Salicylate Solution (Catalogue No. 530-25) in order to determine levels of total plasma salicylate.

Plasma levels of paracetamol and its sulphate, glucuronide, cysteine and mercapturic acid conjugates were determined using high performance liquid chromatography (Adriaenssens & Prescott, 1978). An Orlita pump (Model AE 10-4) was used with a Waters model 440 U.V. absorbance

detector (254 nm filter) and peak areas were measured with a Hewlett Packard HP 3370A integrator. The columns were internally-po lished stainless steel tubes 100 mm x 4.9 mm i.d. slurry packed with Hypersil-ODS (Shandon Southern Products) and fitted with a septum injector. The mobile phase was 0.1 M potassium dihydrogen phosphate, 98% formic acid and isopropanol (100:0.1:1.7 v/v/v). Stock solutions of internal standard/protein precipitant were prepared containing 360 μg/ml of N-propionyl-4-aminophenol in 6% aqueous perchloric acid and were stored deep frozen prior to use. Aliquots of plasma were pipetted into glass tubes which were then placed in a vortex mixer while an equal volume of internal standard/perchloric acid solution was addded slowly. The tubes were centrifuged and up to 5 μl of the clear supernatant injected directly into the column. The assay was calibrated using plasma standards containing paracetamol.

2.6 Electrophysiological experiments in vivo

Experiments were performed on male wistar rats (weight range: 200 - 350 g).

2.6.1 Anaesthesia

Animals were anaesthetized by i.p. injection of urethane (ethyl carbamate, 25% w/v in distilled water) 0.6 ml/100 g body weight.

2.6.2 General

A midline incision was made in the ventral aspect of the neck, and a tracheal cannula was inserted below the level of the larynx. The left carotid artery was cannulated with a nylon catheter (OD 0.75 mm), and arterial blood pressure monitored by connection of the catheter to a pressure transducer (Bell and Howell, 4-442), and the signal subsequently amplified and displayed on a chart recorder (Devices M4). A cannula (OD 0.63 mm) was inserted into the right femoral artery for the injection of drugs at the level of the iliac bifurcation of the abdominal aorta. In experiments requiring intravenous administration of substances, a further cannula (OD 0.75 mm) was inserted into the femoral vein. Body temperature was monitored continuously, and maintained at 37 ± 0.5°C by an electronic heating blanket (Harvard apparatus Ltd).

2.6.3 Dissection

The tibial nerve of the left hindlimb was exposed through a longitudinal incision in the groin and medial aspect of the leg from the pelvis to the sole of the foot. A free exposure of the nerve was obtained by removing the semitendinosis, semimembranosis, biceps femoris and medial gastrocnemius muscles. The tendons of gastrocnemius and flexor digitorum longus were cut about 5 mm proximal to the calcaneum. The plantar nerves were then exposed by careful incision and reflexion of the crural fascia overlying the stumps of the Achilles tendon, and the primary articulo-cutaneous ramus (PACR) (Guilbaud et al., 1985) identified where it leaves the medial plantar. The dissection required to expose the nerve

and to provide sufficient skin flap to form a liquid paraffin pool adequate to cover the exposed nerve during electrical recording, inevitably led to rupture of the cutaneous branches of the PACR so that recording was restricted to activity in nerve fibres innervating the subcutaneous tissues. Figure 2.1 shows a diagramatic representation of the recording arrangement together with a photograph of working preparation.

2.6.4 Recording afferent nerve activity

Electrical activity in afferent nerve fibres was recorded either from fine filaments split from the PACR close to its origin from the medial plantar nerve, or from the intact PACR after it had been dissected away from the adjacent tissues. On a few occasions, recordings were made from fine nerve filaments dissected from the medial plantar nerve proximal to the PACR in order to facilitate single unit recording. The tibial nerve was routinely cut centrally to abolish efferent nerve activity in the PACR. Electrical activity was recorded extracellularly using bipolar platinum-iridium wire electrodes mounted on a micromanipulator, and carefully placed in contact with the lower side of the nerve filament. The electrical signal was amplified (X 1000), and displayed on a storage oscilloscope (Tektronix 5113). The signal was then digitized using a digital audio processor (Sony PCM 701-ES) for storage on a videotape recorder (Sony Betamax SL-HF100 UB). Figure 2.2 illustrates the arrangement used for recording and storing data. The neural information recorded on videotape was monitored by relaying the output signal from the video recorder to another channel of the oscilloscope. The output

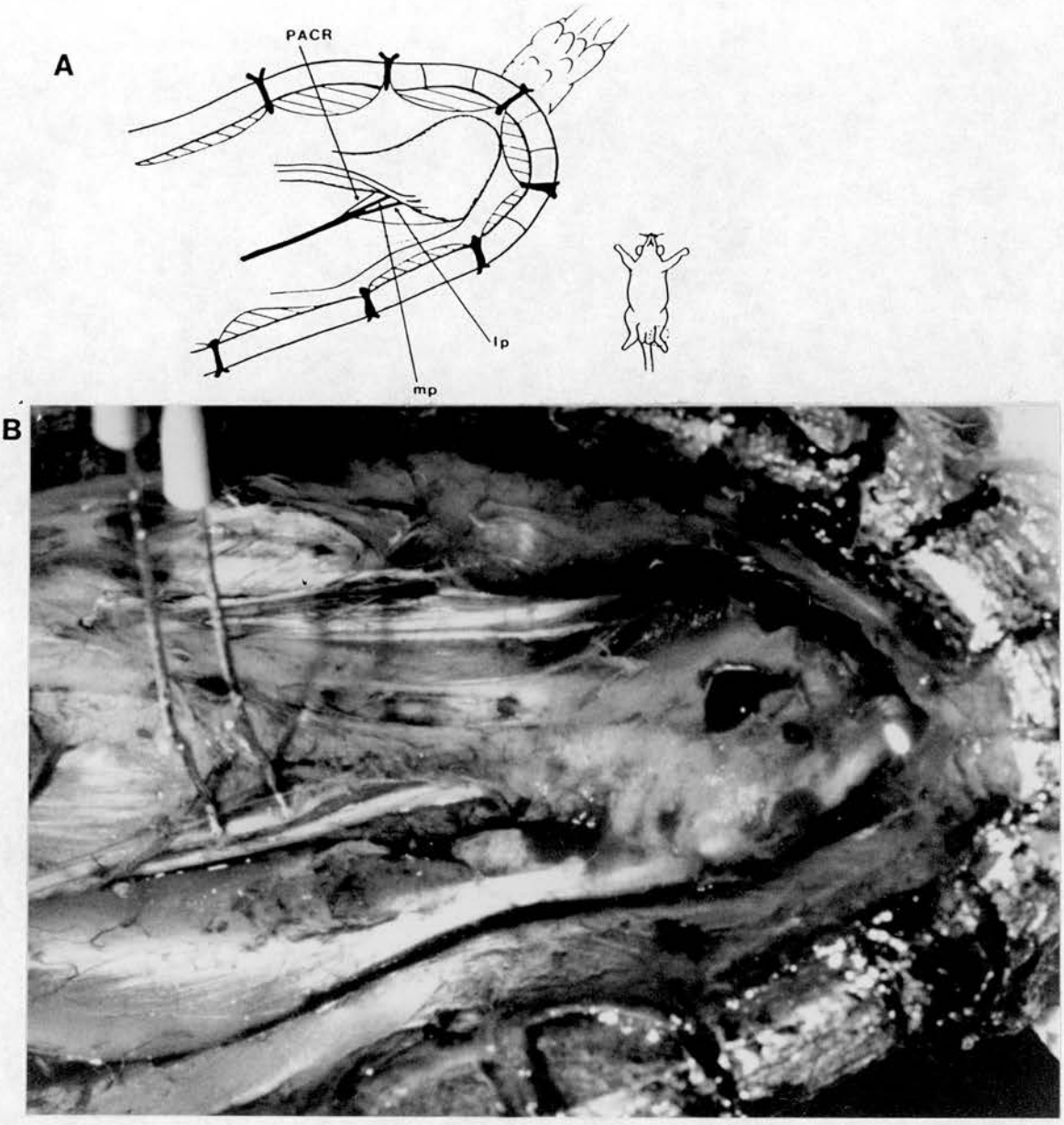


Fig. 2.1 (A) Schematic diagram showing the PACR as prepared for recording purposes. The skin has been dissected from the limb to form a paraffin oil pool, thus exposing the tissues of the ankle joint. Labelled in the diagram is the PACR and the the medial (mp) and lateral (lp) plantar nerves. The PACR leaves the medial plantar nerve and passes under the saphenous nerve to innervate the tissues of the joint. (B) Enlarged (x4) photograph of the working preparation. The PACR is hooked up onto a pair of platinum-iridium wire electrodes for recording afferent nerve activity from the ankle joint.

from the amplifier was also passed through a voltage discriminator (Digitimer), and action potentials of a selected amplitude were counted, converted to an analogue signal proportional to the frequency of discharge, and displayed on the chart recorder (Devices M4). Output from the voltage discriminator was also relayed to a loudspeaker.

Afferent units were found by exploring the exposed medial aspect of the ankle joint with a glass probe of approximately 1 mm diameter while recording electrically from the dissected nerve. The thresholds and properties of the afferent units were determined by using calibrated von Frey hairs. Quantitative mechanical stimuli were delivered using an electromechanical indentation generator (Somedic, Sweden). Ramp and plateau stimulus waveforms were used routinely, with a maximum displacement of 200 - 600 μm. Indentation stimuli were of 2 seconds duration, applied at one or two minute intervals in order to minimise receptor fatigue. The mechanical transducer probe consisted of a silver wire core isolated from a metal cylinder casing of 1mm external diameter. The tip was smooth and rounded, being sealed with plastic resin. The concentric arrangement of the probe allowed its use for localized electrical stimulation at the level of the receptor for identification of mechanosensitive units. Conduction velocity (v) was calculated from the conduction distance (d) measured in situ and the conduction time (t) for the action potential to travel from the stimulating to recording electrodes, where $v = d/t$ (ms^{-1}).

It should be noted that the high level of technical ability required to dissect the PACR for recording purposes resulted in only a proportion of preparations being functional. On average a successful recording was obtained in 60% of the preparations.

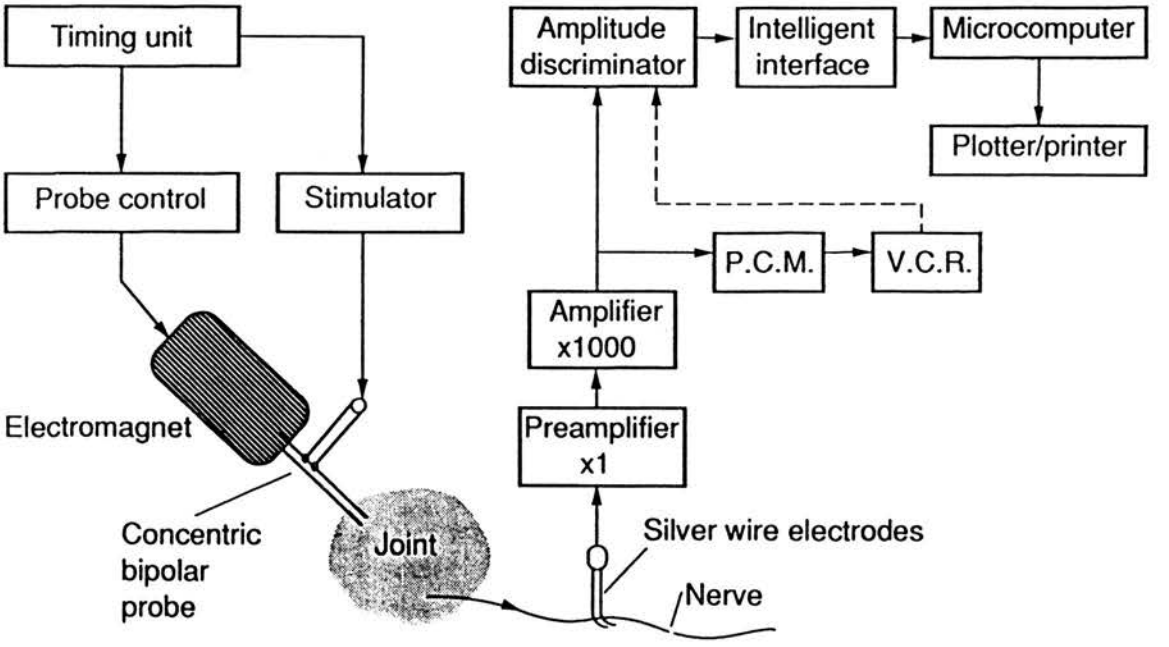


Fig. 2.2 Schematic diagram showing the arrangement of apparatus used for the application of mechanical stimuli, for electrophysiological recording and in the collection and storage of data.

2.6.5 Drug administration

Intra-arterial (i.a.) injections were generally made in a volume of 0.1 ml into the bifurcation of the abdominal aorta via the cannula inserted anterogradely into the right femoral artery, and washed in with 0.2 ml saline (0.9 % w/v aqueous NaCl). Where combination injections were made using more than one drug a volume of 0.2 ml was used followed by a 0.2 ml saline wash. Injections were completed within a period of 1 s. At least ten minutes were allowed to pass between injections, or longer if the drug under investigation was known to induce tachyphylaxis. Other drugs were injected i.v. into the femoral vein in an appropriate volume, and the catheter flushed with 0.2 ml saline.

Control injections of 0.1 ml saline i.a. were made and the catheter flushed with 0.2 ml saline. These injections were made throughout each experiment, and served as a control to determine the effect upon afferent discharge of the drug vehicle and of the injection procedure. In all cases injection of saline had no effect on the receptors under study.

2.6.6 Drugs used

Most of the drugs used dissolved in saline, although capsaicin was dissolved in a solution of 10% tween 80, 10% absolute ethanol and 80% saline at a concentration of 1 $mgml^{-1}$, and PGI_2 was dissolved in Tris buffer pH 10 at a concentration of 50 $\mu g/ml$. Dilutions prior to injection were made in saline equilibrated at room temperature or in the case of PGI_2, Tris buffer pH 8.

The drugs used, their sources, and details of their molecular weights are to be found in the Appendix I. Doses referred to in the text are those of the salt where applicable.

2.6.7 Data analysis

The output of the video tape recorder containing the electrical recording was passed through an amplifier and filter module (band pass 50 - 300 Hz) and fed to a spike processor (Digitimer) permitting the counting of selected action potentials. Each action potential that fell within the window generated a pulse of 1 ms duration. The number of inpulses occurring in 0.1 s intervals was collected and stored by a personal computer (IBM PC) programmed to collect over a period of up to 900 s (Software written and kindly supplied by Dr M.Dutia, Dept. Physiology, University of Edinburgh). A marker was used to indicate the the point at which a drug injection was made. Collected data were stored on floppy disc, and subsequently analysed using the same programme on the personal computer (see Figure 2.3).

A computer-drawn plot of the action potential discharge frequency as a function of time was displayed on the computer monitor, from which discharge was analysed in the pre-injection control period and, thereafter during defined intervals following injection of the drug. When analysing the effects of a drug, the control period was defined as the 10 - 60 s period immediately prior to injection. Responses to mechanical stimuli were expressed as the number of action potentials produced per indentation stimulus.

The defined time period (t seconds), for which the computer was required to process discharge frequency, was selected using a moving cursor which directed the computer to the memory-stored data displayed on the monitor. Drug effects were quantified either as the peak discharge obtained over the defined time interval, or, as with prolonged drug effects, the total number of action potentials produced above the control rate of discharge. The calculation shown below was used to determine the number of action potentials evoked following a drug injection:

i) Σx: the total impulses (number of action potentials) occurring in the period t seconds, for control and for test periods. Units: impulses.

ii) $\overline{x}$ i.p.s.: the mean impulses per second occurring in each period t seconds.

Hence $\overline{x}$ (control) = Σx (control)/t (control),

and $\overline{x}$ (test) = Σx (test)/t (test)

Units: impulses per second (i.p.s.).

iii) delta x: the difference in the mean discharge occurring in test and control periods.

Thus, delta $\overline{x}$ = $\overline{x}$ (test) - $\overline{x}$ (control). Units: i.p.s.

iv) delta Σx: the absolute difference of discharge from control levels, given by: delta Σx = Σx (test) - [$\overline{x}$ (control) x t (test)].

Units: i.p.s.

Of the above, iii and iv will have negative values when there is a depression of discharge. i and ii may be equal to zero. Data integrated with respect to control (i.e. delta Σx) take into account the two-dimensional character of responses, and both the magnitude and the duration of the induced change in discharge are included in the analysis of the response (see McQueen, 1977). This parameter measures the increase or decrease in discharge in the test period relative to the pre-injection discharge which, it is assumed, would have remained constant in the absence of any modifying effect of the drug. This assumption is of course supported by the finding that control injections of saline had no effect on the receptors under study.

Whenever possible, dose response studies were performed and an appropriate parameter expressing the response was plotted against $\log_{10}$ dose.

2.7 Electrophysiological experiments in vitro

Experiments were performed on male wistar rats (weight range 250 - 350g).

2.7.1 General

Rats were anaesthetized with urethane (25 % w/v, 0.6 ml/100 g), and the femoral artery of the left hindlimb was cannulated anterogradely using a nylon catheter (OD 0.63 mm). Heparinized saline (500 units/kg in 3ml saline) was flushed through the limb via the arterial cannula following section of the femoral vein to allow the escape of blood and perfusate.

The limb was then perfused with pre-warmed (32°C), oxygenated (95 % O2 / 5 % CO2) Krebs solution (NaCl 118.4 mM, KCl4.7 mM, MgSO4.7H2O 1.2mM, KH2PO4 1.2 mM, NaCO3 25 mM, CaCl2 2H2O 2.5 mM, Glucose 11.1 mM) at a rate of 2 ml/min using a peristaltic pump (Watson Marlow, 101U). Perfusion pressure was monitored continually by connection of the catheter to a pressure transducer (Bell and Howell, 4-442) via a T-piece connector and the subsequent signal displayed on a chart recorder (Lectromed, MX6).

2.7.2 Dissection

The left hindlimb was surgically removed from the animal by cutting through the muscle and bone of the upper thigh. The animals were then killed by cardiac puncture. The isolated limb was placed into a perspex superfusion bath containing pre-warmed oxygenated Krebs solution (composition as described above for perfusion fluid). The superfusion fluid was allowed to flow from the reservoir and through the bath at a rate of 5 $mlmin^{-1}$. The paw of the limb was secured in the bath on a perspex platform, and all skin was carefully removed from the leg to expose the underlying tissues. Access to the tibial nerve and subsequent dissection of the PACR for recording purposes were as described above for the <u>in vivo</u> preparation. The desheathed nerve was cut centrally, and placed in a small perspex recording chamber filled with liquid paraffin where recording of afferent nerve activity was achieved using a monopolar platinum-iridium wire electrode. Figure 2.3 shows a diagramatic representation of the preparation.

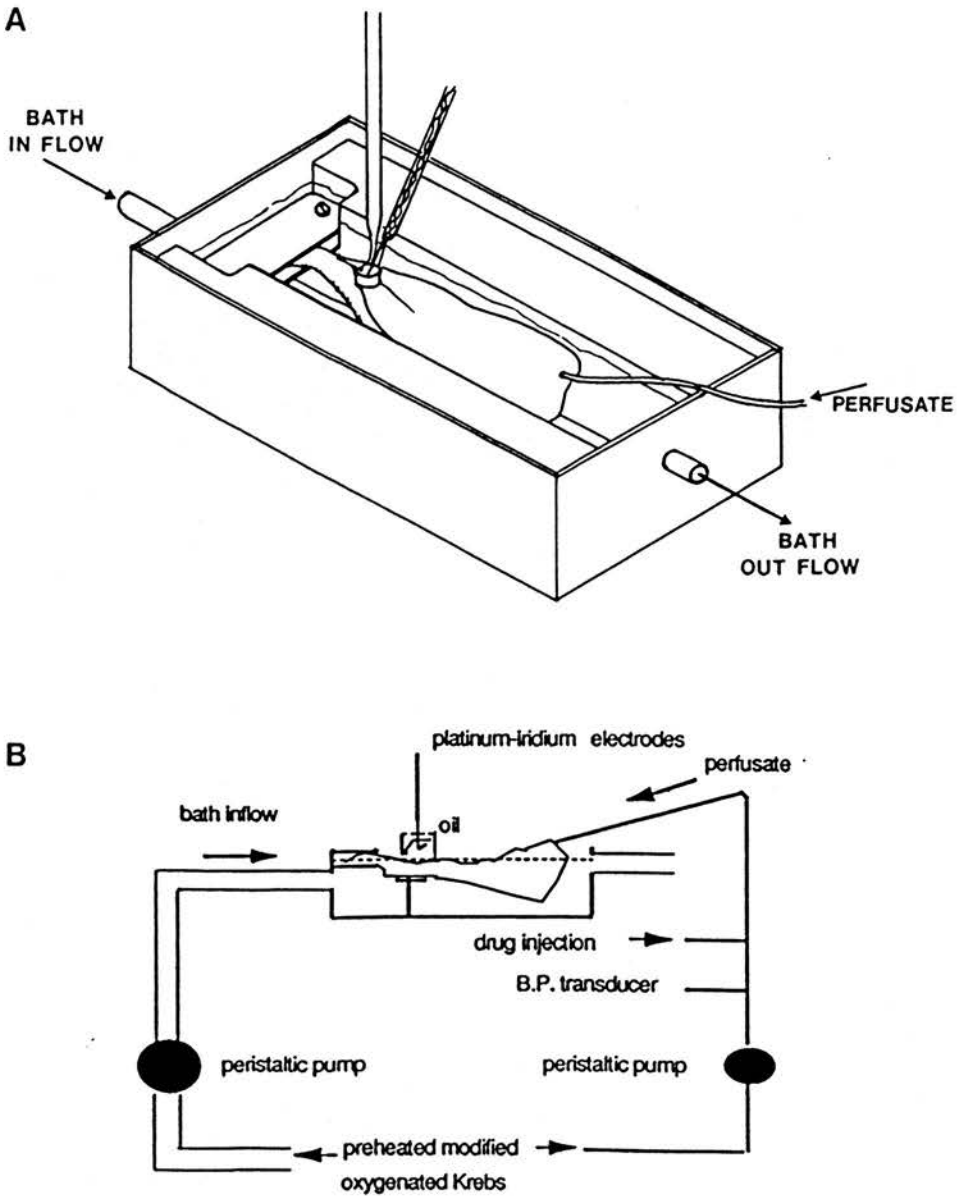


Fig. 2.3 Schematic diagram showing the isolated hind-limb as prepared for electrophysiological recording from the PACR. (A) The limb has been removed and placed in a perspex superfusion bath following cannulation of the femoral artery for perfusion. The limb is secured in the bath by a perspex clamp over the paw. Electrophysiological recordings are made using a small bath filled with liquid paraffin and positioned over the PACR (B) Diagramatic layout of the perfusion/superfusion apparatus.

2.7.3 Recording afferent nerve activity

Afferent nerve activity was recorded from the desheathed PACR using monopolar platinum-iridium wire electrodes mounted on a micromanipulator and placed inside the recording chamber. The proximal, cut end of the nerve was carefully wound around the electrode to obtain electrical contact. The original signal was amplified (X 10,000. Digitimer, Neurolog) and displayed on an oscilloscope (Tektronix 5103N). The amplified signal was then digitized using a digital audio processor (Sony PCM 701-ES) for storage on a videotape recorder (Sony Betamax SL-HF 100 UB). The output from the amplifier was also passed through a voltage discriminator (WPI model 120).

Action potentials of a selected amplitude were counted and converted to an analogue signal proportional to the frequency of discharge, which was displayed on the chart recorder (Lectromed, MX6).

Afferent units were found by exploring the exposed medial aspect of the ankle joint with a plastic probe of approximately 1 mm diameter while recording electrically from the dissected nerve. The thresholds and properties of the afferent units were determined by using calibrated von Frey hairs. A concentric bipolar electrode was used to produce localized electrical stimulation at the level of the receptor. Conduction velocity (v) was measured from the conduction distance (d) in situ and the conduction time (t) from stimulating to recording electrodes, where $v = d/t$ (ms^{-1}).

As described for <u>in vivo</u> electrophysiological studies the high level of technical ability required to dissect the PACR for recording purposes resulted in only a proportion of preparations being functional. On

average 64% of the preparations could be used in experiments, in the remaining preparations a successful recording was not obtained.

2.7.4 Drug administration

Drugs were generally administered intra-arterially (i.a) into the perfusate as a bolus injection of 0.1 ml followed by a 0.2 ml saline wash. Combination injections of more than one drug were given in volumes of 0.2 ml with a 0.2 ml wash. A period of at least ten minutes was allowed between drug injections, or longer if the drug under investigation was known to cause tachyphylaxis. Other drugs were added to the perfusion and superfusion fluid in order to produce a sustained effect.

In each experiment control injections of 0.1 ml saline, followed by a further 0.2 ml saline served as a control, to determine the effect of the drug vehicle on afferent discharge.

2.7.5 Drugs used

Most of the drugs used dissolved in saline, although capsaicin was dissolved in a solution of 10% tween 80, 10% absolute ethanol and 80% saline at a concentration of 1 $mgml^{-1}$, and $PGF_{2\alpha}$ which was obtained as a commercial solution (Lutalyse). Dilutions were made in saline equilibrated to room temperature prior to injection.

The drugs used, their sources and details of molecular weights and structures are given in Appedix I. Doses stated in the text are those of the salt where applicable.

2.7.6. Data analysis

Data analysis was carried out as described previously for the *in vivo* electrophysiology.

2.8 Isolated nerve preparations

2.8.1 Preparation of tissues

Adult New Zealand White (NZW) rabbits were killed using a captive bolt device, and male hooded rats weighing 200-300 g were stunned by a blow to the head and killed by cardiac puncture. Segments of rabbit cervical vagus, rat saphenous nerve or a motor branch of sciatic or tibial nerve approximately 10 - 15 mm in length were dissected out bilaterally and placed in modified Krebs solution (NaCl 122 mM, KCl 4.4 mM, MgCl 1.2 mM, CaCl 1.3 mM, $NaHCO_3$ 15.4 mM, KH_2PO_4 1.2 mM, glucose 5.5 mM) oxygenated with 95% O_2 / 5% CO_2, and containing 3 μM indomethacin, at room temperature. The connective tissue sheath around each isolated vagus nerve was then carefull y removed using watchmaker forceps.

2.8.2 Extracellular recording

Within one hour of dissection, 10 - 15 mm sections of desheathed nerve were transferred to two compartment perspex baths to permit extracellular recording of prostanoid-induced membrane potential changes (fig. 2.4). Each nerve was positioned so that approximately half of its length lay in the first compartment, while the remainder projected

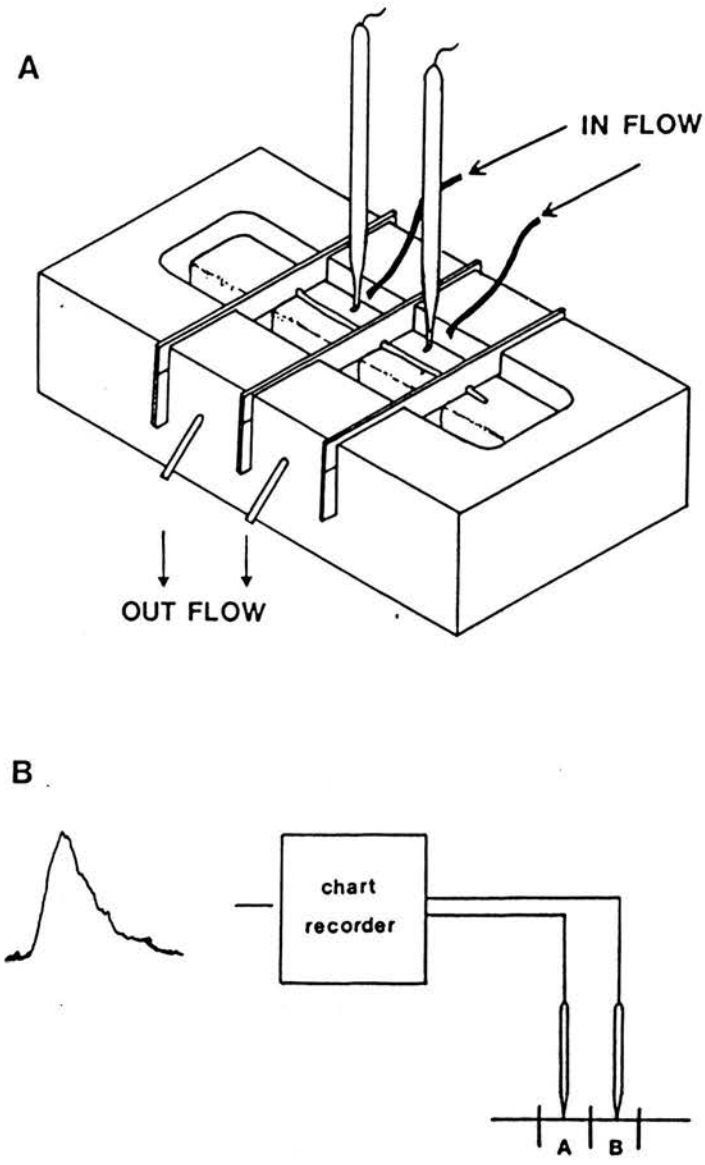


Fig. 2.4 Apparatus for recording extracellularly from isolated nerves. (A) Diagram of the three chambered bath. (B) Layout of recording apparatus. The continuous chart record displays the whole nerve polarization when recording differentially from chambers A and B.

through a greased slot (Dow-Corning high vacuum grease) into the second. The DC potential between the two compartments was recorded via silver-silver chloride electrodes connected to the tissue preparation via agar-saline / filter paper bridges and was displayed on a potentiometric chart recorder (Rikadenki). Each compartment of the bath was perfused with oxygenated Krebs solution containing 3 μM indomethacin to inhibit endogenous prostanoid production, the perfusate being dripped directly on to the tissues. Drugs were applied at a known concentration via the superfusion stream into the first compartment only. The temperature of each preparation was maintained at 27 ± 1°C. A diagramatic representation of the preparation is shown in figure 2.4.

2.8.3 Design of experiments

Concentration-response curves were constructed using non-cumulative, sequential applications. For all of the natural prostanoids, contact times were of sufficient duration for the evoked potential change to have stabilized (3 minute contact time). Tissues were allowed to repolarize fully between application of each concentration of drug. The maximum and ED50 of each concentration response curve was estimated by computer-aided fit of a logistic curve to the experimental data (see Barlow, 1983).

To examine the possibility that the drugs used caused tachyphylaxis, non-cumulative, sequential concentration-effect curves were constructed, using graded applications of either low to high concentrations or high to low concentrations of drug. Using this protocol, concentration-effect curves were constructed using PGE_2, PGE_1,

PGI_2 and cicaprost. A significant shift to the right of the concentration effect curve for a particular agonist when going from high concentration to low concentration was regarded as an indication of tachyphylaxis following the application of high doses. Results between tissues were compared by testing their responsiveness to a standard supramaximal concentration of PGI_2, and expressing responses as a percentage of the PGI_2 maximum. Tissue viability was determined using a standard depolarizing concentration of carbachol both before and after construction of prostanoid concentration-effect curves.

Investigations into the role of cyclic AMP in prostanoid-induced depolarization of the rabbit isolated vagus nerve involved the addition of forskolin, 8-Bromo cAMP and IBMX to the superfusion medium for various periods of time and at different concentrations for the construction of a sequential concentration-response curve.

2.8.4 Drugs and solutions

The naturally-occurring prostanoids, PGD_2, PGE_2, PGE_1, $PGF_{2\alpha}$, the selective EP agonists AH13205, rioprostil, and sulprostone, the synthetic TXA_2-mimetic U46619, the synthetic $PGF_{2\alpha}$ mimetic ICI81008, and the synthetic PGI_2 analogues, iloprost and cicaprost, were dissolved in saline. Dilutions were made using modified Krebs medium to give final concentrations of 1×10^{-10} to 1×10^{-3} M. These were made immediately prior to application. PGI_2 was dissolved in Tris buffered saline (pH 9) at 1×10^{-2} M, dilutions in Tris buffer (pH 8) to 1×10^{-3} M, and further dilutions in modified Krebs medium being made immediately prior to application. Forskolin, 8-Bromo cAMP and IBMX were dissolved in

modified Krebs medium, with dilutions being made immediately prior to use. All drug solutions were kept on ice.

SECTION III

LOCALIZED ADJUVANT-INDUCED MONOARTHRITIS AS A MODEL FOR THE NEUROPHARMACOLOGICAL STUDY OF CHRONIC INFLAMMATION IN RATS

SECTION III

LOCALIZED ADJUVANT-INDUCED MONOARTHRITIS AS A MODEL FOR THE NEUROPHARMACOLOGICAL STUDY OF CHRONIC INFLAMMATION IN RATS

3.1 INTRODUCTION

Ajuvant-induced polyarthritis in the rat is the most widely used animal model for the study of arthritis (see Billingham, 1983) and chronic pain (see Colpaert, 1987). The pathological characteristics of the disease have been described extensively (Pearson & Wood, 1959; Ward & Jones, 1962; Jones & Ward, 1963; Pearson, 1963; Rosenthale & Capetola, 1982; Rainsford, 1982), and knowledge of the disease is substantial.

Adjuvant polyarthritis is a whole-animal disease affecting multiple joints, eyes, ears, nose, tail and penis of the rat. The most severe inflammation occurs in the distal joints of the hindlimbs where, following prolonged leukocyte infiltration of the synovium, there is progressive destruction of the bone, damage to tendons, and loss of cartilage. The extensive systemic nature of the disease is marked by enlargement of the lymph nodes, impairment of liver metabolism and changes in serum and tissue chemistry.

The use of adjuvant polyarthritis in studies examining the immunopathology of various arthritides is perhaps justified. However, for the study of nociceptive mechanisms in chronic inflammation the use

of such a gross disease is unnecessary and may even complicate the examination of changes in nociceptive systems. It would be of great advantage, on both scientific and ethical grounds, if new models could be developed that did not produce severe systemic complications.

This study was undertaken using a novel model of adjuvant-induced monoarthritis to determine whether this less severe, and more localized form of adjuvant arthritis could be used as an alternative to adjuvant polyarthritis for neuropharmacological studies on chronic inflammation in rats. Examination of electrophysiological, behavioural and histopathological characteristics were included in the study. The use of a unilateral model of arthritis further provides the opportunity for using the contralateral normal joint as a within animal control. In electrophysiological studies both limbs cannot be studied readily in vivo. For this reason an in vitro joint preparation was developed and assessed for its use in studies of this type.

3.2 RESULTS

A localized monoarthritis was induced by the injection of low dose Freunds Complete Adjuvant (F.C.A.) as described in the Methods Section.

3.2.1 Electrophysiology - in vivo

Afferent units in the PACR of the tibial nerve were examined in twenty six normal and twenty six arthritic rats. High-threshold slowly adapting mechanoreceptors with receptive fields located in the joint tissues on

the medial aspect of the ankle were examined for their responsiveness to mechanical indentation. Responsiveness to joint flexion or extension could not be examined in the present study as the limb was immobilized to facilitate neural recording. Afferent units with mechanosensitive receptive fields on tendons, fascia and blood vessels were not included in this study.

3.2.1.1 Normal joints

Receptive fields of slowly adapting mechanoreceptors were identified using a blunt perspex probe with a tip diameter of 1 mm to apply localized pressure to the joint surface. Repeated application of pressure stimuli were used to separate true receptive fields from those afferents being excited by damage to their axon (Guilbaud et al., 1985). From the twenty six rats, fifty four slowly adapting units with receptive fields in the joint tissues were identified. Mechanosensitive receptive fields were punctate, ranging from 1 - 2 mm in diameter. Individual units were represented by only a single identifiable receptive field. Mechanical activation thresholds, measured using a series of calibrated von Frey hairs, were generally high and ranged from 7 mN - >90 mN. Values are illustrated in figure 3.1 together with measurements made in arthritic joints. The high thresholds of these receptors often made them difficult to find, compression of the tissues against the underlying bone being the only effective stimulus.

Measurements of conduction velocities were made for thirty eight units and ranged from 0.5 - 16.6 ms^{-1} (mean: 3.45 ± 0.7 ms^{-1}). A low level of spontaneous resting discharge of 0.38 ± 0.06 i.p.s. was present in

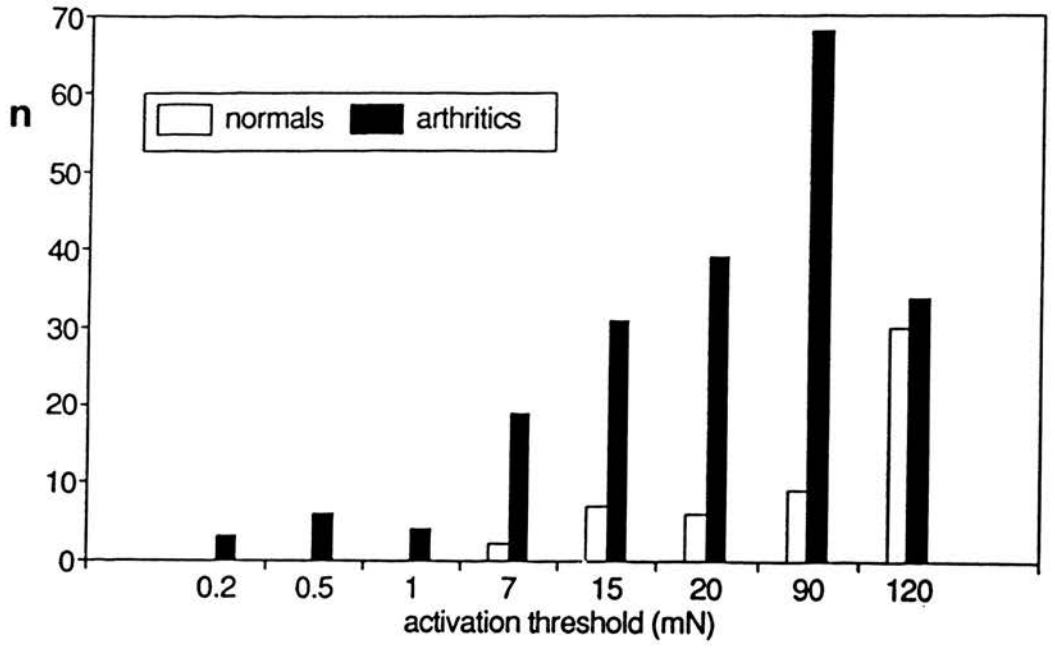


Fig 3.1 Activation thresholds, measured with von Frey hairs, for mechanical stimulation of joint capsule receptors in normal (open bars, n=26) and arthritic (filled bars, n=26) rats.

sixteen (42%) of these units. Mechanical stimuli of ramp and plateau waveform were applied using an electromechanical indentation generator. Phases of dynamic and slowly declining discharge were produced. Repetition of mechanical stimuli led to a steady decline in the unit response over a period of time proportional to the rate of stimulation. These response characteristics are similar to those described previously for this type of receptor by Guilbaud et al. (1985).

As well as mechanosensitive units a low level of background activity was also seen in units for which no mechanosensitive receptive fields could be found by probing in the joint tissues. These afferent units had action potential spike shapes similar to those of identified C fibre afferents. This similarity is based on criteria of action potential spike height (Heapy et al., 1987) and spike width. Action potentials generally had amplitudes of below 30 μV and spike widths of 2 - 3 ms.

3.2.1.2 Arthritic joints

Rats were used for experiments between 12 and 35 days following the injection of adjuvant around the left ankle joint. Dissection of the lower limb for recording purposes revealed the presence of large amounts of fibrous tissue, which bound together the inflamed muscles, tendons, fascia and skin. The capsular tissues around the tibio-tarsal joint were markedly enlarged and thickened.

From twenty six rats a total of two hundred and four slowly adapting mechanoreceptors with receptive fields in the joint tissue were identified. This figure is approximately three times greater than that for normal joints (see fig. 3.2). In contrast to the normal state,

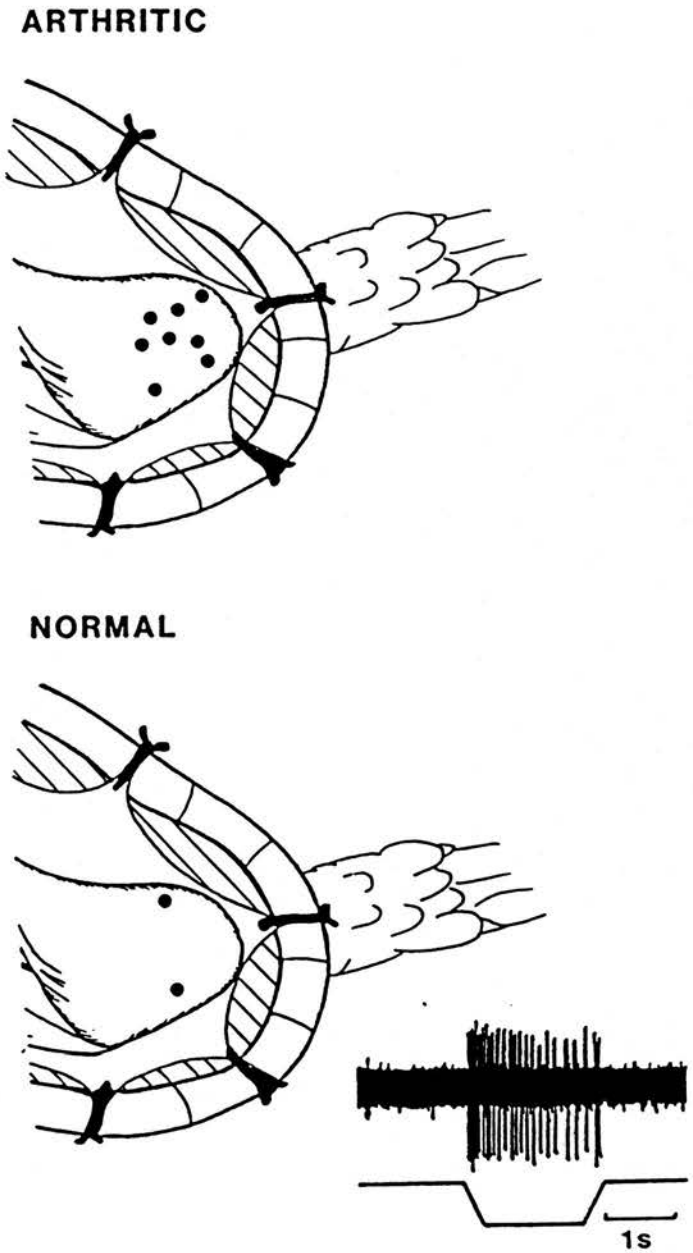


Fig 3.2 Diagram illustrating the distribution of punctate receptive fields for slowly adapting mechanoreceptors in capsular tissue of representative arthritic and normal ankle joints. Each receptive field corresponds to a single afferent unit. The inset shows a neurogram of a typical slowly adapting response of an articular high-threshold slowly adapting mechanoreceptor.

receptors in the arthritic joints were activated by the application of light pressure. The mechanical activation thresholds ranged from 0.2 - >90 mN. The lower threshold units found in inflamed joints were not seen in those from normal joints (fig. 3.1). Individual units were represented by only a single receptive field, each being between 1 and 2 mm in daimeter as seen in normal joints.

Measurements of conduction velocity were made for thirty nine units and ranged from 0.3 - 4.6 ms^{-1} (mean: 1.48 ± 0.3 ms^{-1}). A mean rate of ongoing discharge equal to 1.5 ± 0.32 i.p.s. was present in thirty two (82%) of these units. Thus, there were on average twice the number of active units in arthritic joints, and their mean rate of discharge was approximately four times greater than that in recordings from normal joints.

Application of the mechanical probe produced pitting of the tissues, and generally responses to repeated stimuli were more susceptible to fatigue than in normal joints, as described previously by Guilbaud et al. (1985) in rats with adjuvant polyarthritis.

Units for which no mechanosensitive receptive fields could be found had a high level of resting discharge and were more numerous than in recordings from normal joints. These units had action potential spike shape characteristics similar to those of identified C fibre afferents as described above for normal joints.

3.2.2 Electrophysiology - in vitro

3.2.2.1 Normal joints

Following surgical removal, the hind limb was simultaneously perfused and superfused with pre-warmed, oxygenated Kreb's solution as described in the Methods (Section II). No obvious tissue oedema occurred in the tissues around the ankle joint for perusion periods of up to eight hours. Receptive fields of slowly adapting high-threshold mechanoreceptors were identified as described for in vivo studies. From eight rats, fourteen units with receptive fields in the joint tissues were identified. Receptive fields were punctate and of between 1 and 2 mm in diameter. Mechanical activation thresholds were generally high, and similar to those found in vivo. Values ranged from 10 - > 100 mN as shown in figure 3.3. Receptive fields were often difficult to find and activation generally required that the tissues be compressed against the underlying bone as found in vivo.

Measurements of conduction velocity were made for six units and ranged from 0.4 - 2 ms^{-1} . A low level of spontaneous resting discharge of 0.6 ± 0.1 i.p.s. was present in two (33%) of these units. Using a blunt tipped perspex probe, manual application of mechanical stimuli evoked slowly adapting responses similar to those seen in vivo. Mechanical responsiveness was maintained for the duration of all recordings, which in some cases covered periods of up to eight hours.

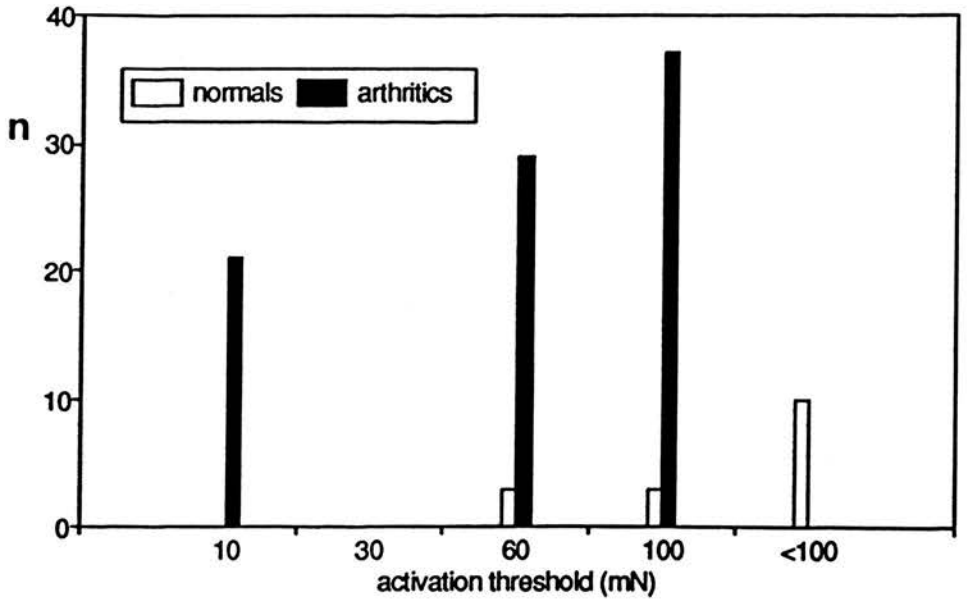


Fig 3.3 Activation thresholds, measured with von Frey hairs, for mechanical stimulation of joint capsule receptors in normal (open bars, n=8) and arthritic (filled bars, n=8) rats in vitro.

3.2.2.2 Arthritic joints

Rats were used for experiments as described above for in vivo studies. From eight rats, eighty seven slowly adapting mechanoreceptors with receptive fields in the joint tissues were identified. This figure is approximately six times greater than that for normal joints. Receptors could be activated by the application of light pressure. Mechanical thresholds ranged from 10 - > 100 mN, with the majority of units having thresholds of 90mN or below as illustrated in fig 3.3.. Receptive fields were of similar size to those seen in normal joints, ranging from 1 - 2 mm in diameter.

Measurements of conduction velocity were not made in these experiments, all units having action potential spike shape characteristics similar to those of identified C fibre afferents. A mean rate of ongoing discharge equal to 1.2 ± 0.2 i.p.s. was present in two (66%) of three units that were examined in detail. From these figures there were approximately twice the number of active units in arthritic joints, and their rate of ongoing discharge was double that seen in normal joints.

3.2.3 Behaviour and gross pathology

Injection of adjuvant subdermally around the left ankle joint produced swelling of the tissues within a few hours. The initial swelling and inflammation continued for four or five days before beginning to subside. From day eight a secondary inflammation developed and reached a plateau within two days. This secondary phase continued for the duration

of the experiment, during which time the right hindlimb was unaffected.

Measurements of ankle circumference illustrated in figure 3.4 show that a marked swelling of the joint developed from day one post-injection and increased gradually to reach a stable level from day six onwards. The circumference of the contralateral joint showed only the normal increase in size seen in non-injected rats.

The rate of weight gain were slightly reduced in arthritic rats compared to controls for the first eight days. A normal rate was achieved from this time onwards although the weights of arthritic rats never approached those achieved by controls (fig. 3.5).

Standing and walking weight load scores for the injected hindlimb increased markedly from day one following adjuvant administration. The scores declined slightly following the initial reaction, but remained elevated throughout the test period (figs. 3.6 - 3.7). Scores for the right hindlimb of arthritic animals remained at basal levels as did those for both hind limbs of control animals.

As illustrated in figure 3.8, pressure thresholds for paw withdrawal were reduced markedly following injection of adjuvant. Reductions in thresholds became significant two days after treatment and remained lowered for the duration of the study. However, withdrawal thresholds for the uninjected paws displayed similar reductions with the same time course. Thresholds for control rats also displayed a significant reduction at one time point during the test period.

Thresholds to mechanical indentation stimuli were reduced from day one onwards for the arthritic limbs of treated rats (fig. 3.9). No change in thresholds was observed for uninjected limbs or in either foot of control animals.

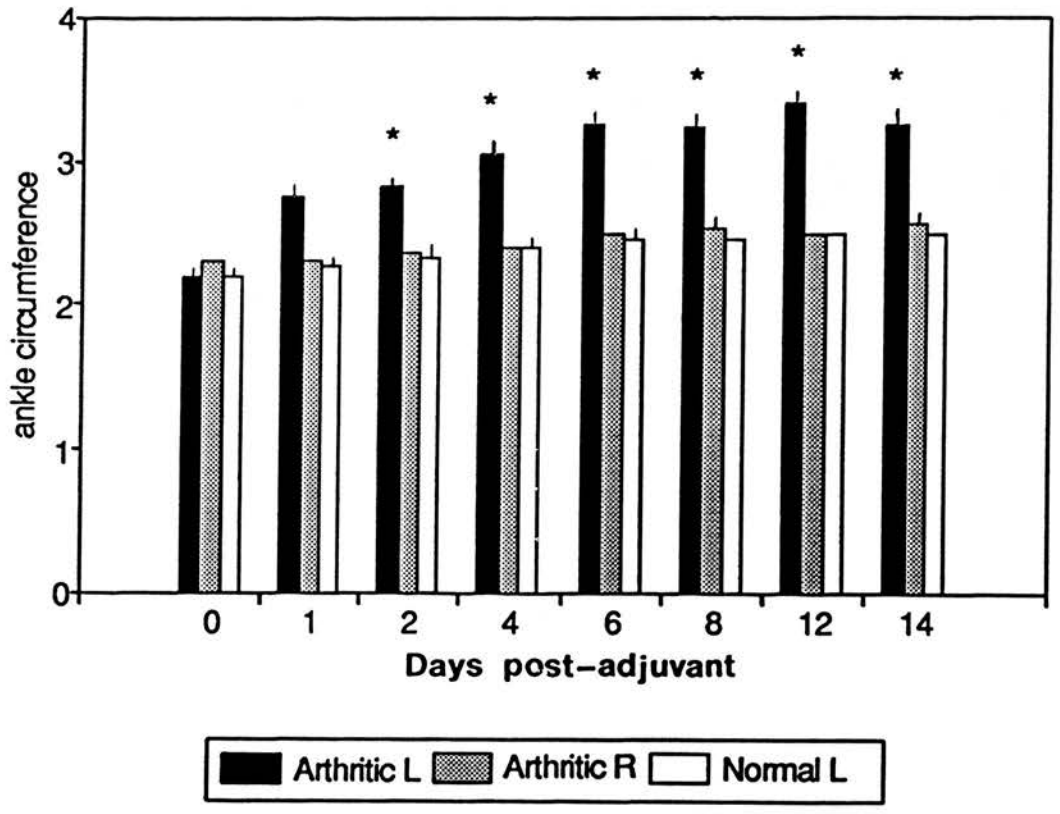


Fig 3.4 Changes in ankle circumference resulting from injection of F.C.A. around the left ankle joint on day zero. Bars represent mean values for measurements from the injected left ankle (L, closed bars, n=4), contralateral non-injected ankle (R, shaded bars, n=4), and the left ankle (L, open bars, n=4) of control rats. The vertical lines above each bar represent the s.e.m.. Significantly greater mean values are shown as * when $p < 0.05$ (Wilcoxon).

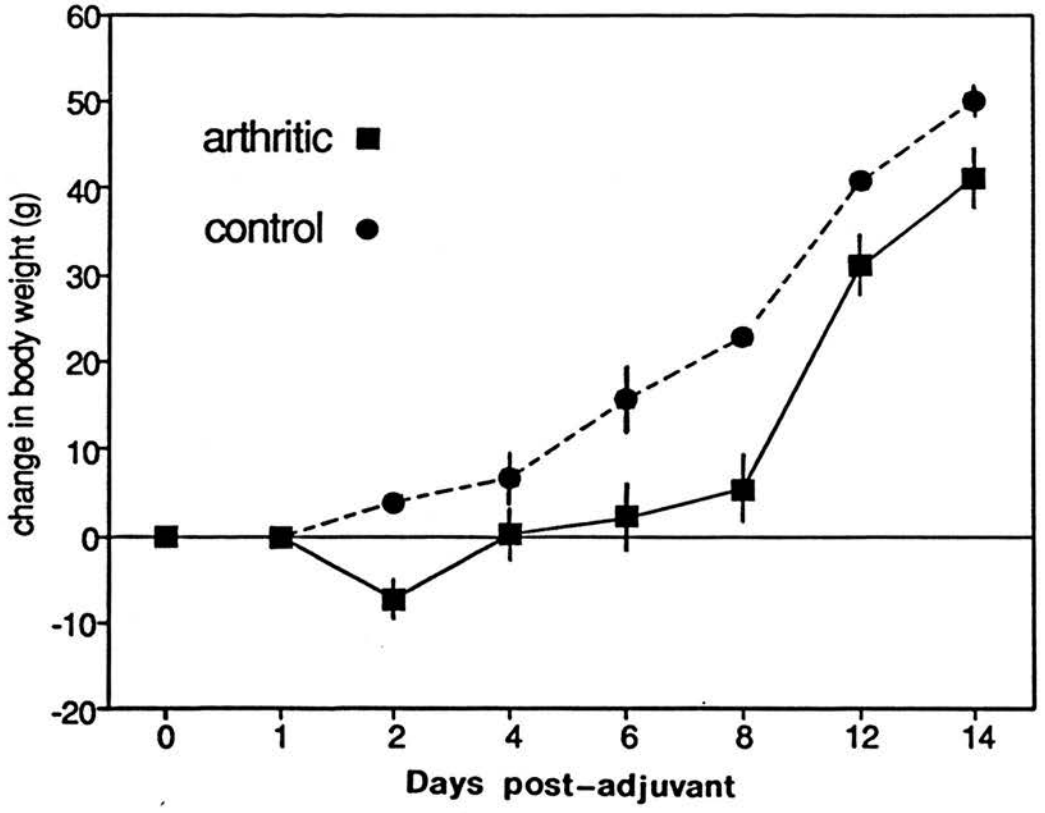


Fig 3.5 Weight gain over the 14 day period following injection of F.C.A. on day zero. No significant differences in weight gain were seen for control (n=4) and and arthritic (n=4) rats. Control values are for normal rats.

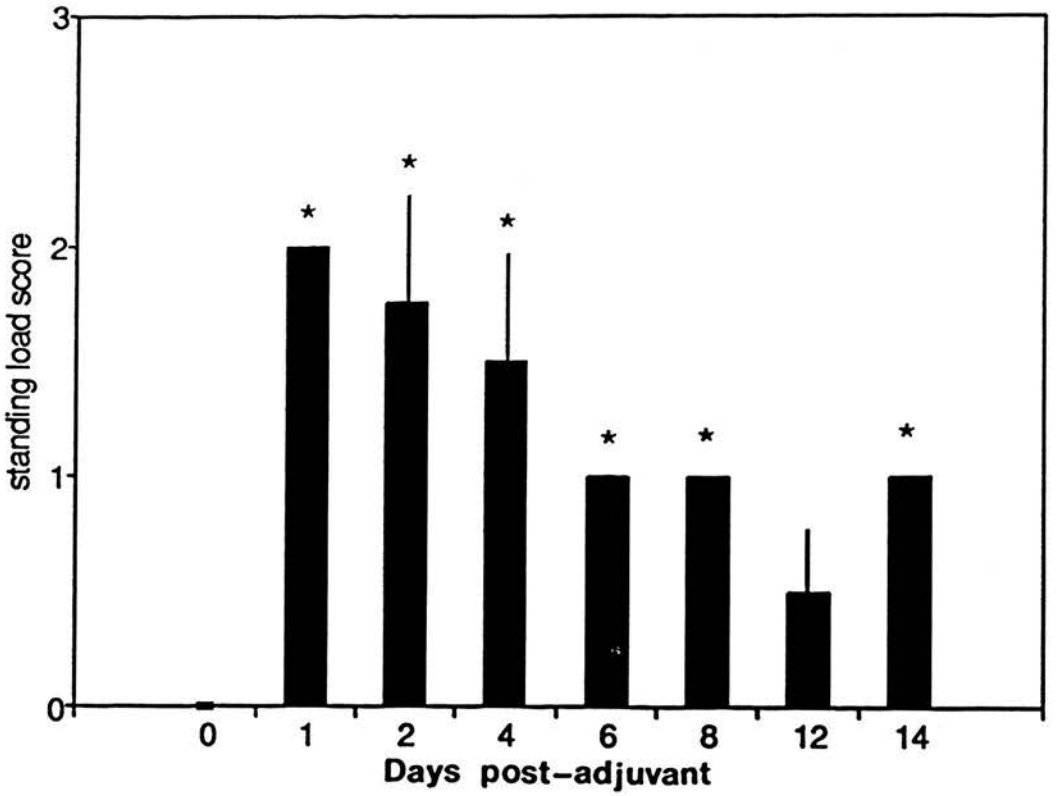


Fig 3.6 Mean standing load scores for the left hindlimb of of arthritic rats (n=4) for the 14 day period following injection of F.C.A.. Mean scores which are significantly greater than those for control rats are shown as * when $p < 0.05$ (Wilcoxon).

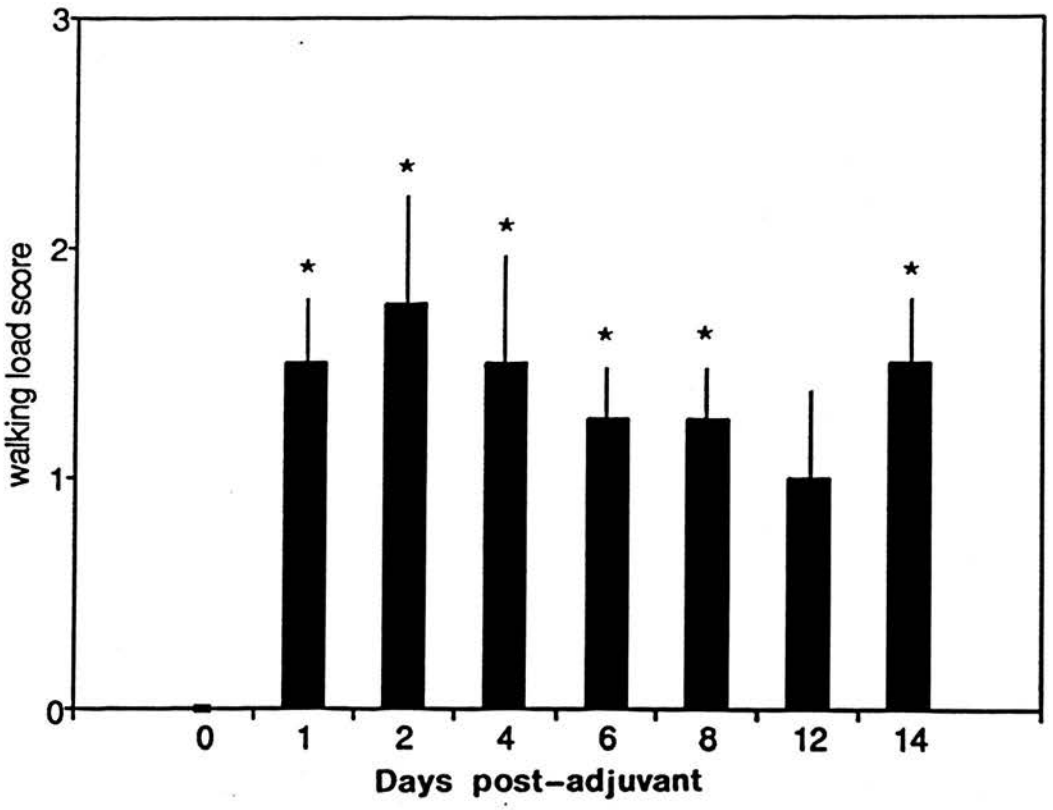


Fig 3.7 Mean walking load scores for the left hindlimb of of arthritic rats (n=4) for the fourteen day period following injection of F.C.A.. Mean scores which are significantly greater than those for control rats are shown as * when $p < 0.05$ (Wilcoxon).

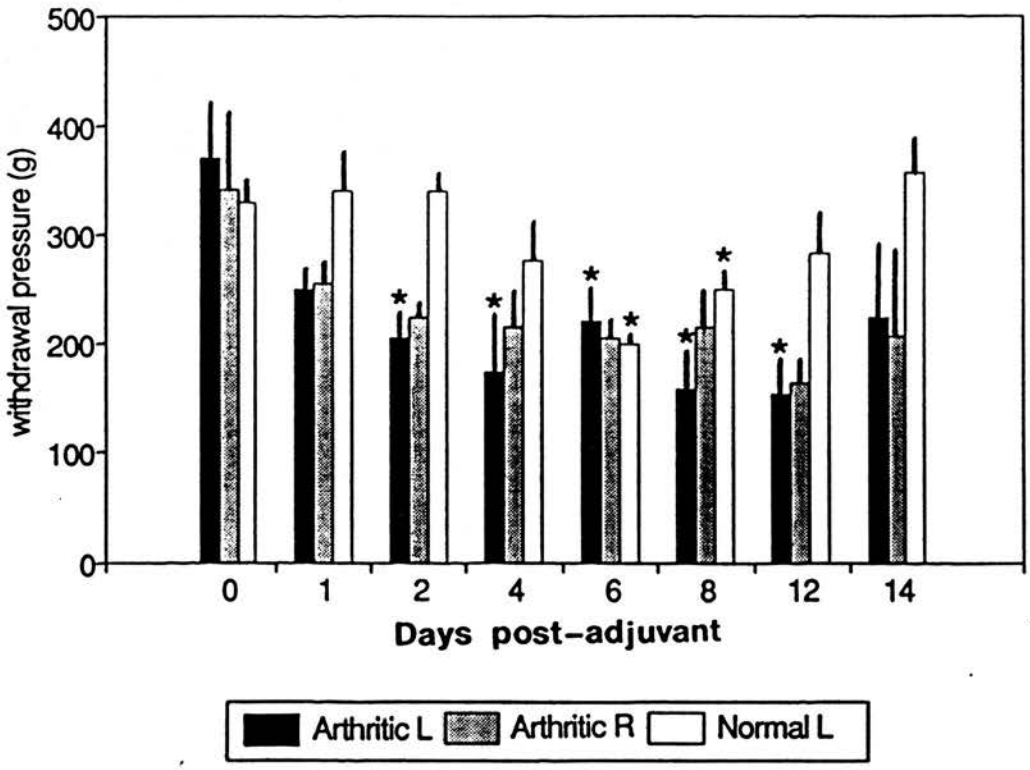


Fig 3.8 Mean withdrawl thresholds to graded pressure stimuli over the 14 day period following injection of F.C.A. around the left ankle joint. Bars represent mean values for measurements from the injected left ankle (L, closed bars, n=4), contralateral non-injected ankle (R, shaded bars, n=4), and the left ankle (L, open bars, n=4) of control rats. The vertical lines above each bar represent the s.e.m.. Mean thresholds significantly lower than at day zero are shown as * when $p < 0.05$ (Wilcoxon).

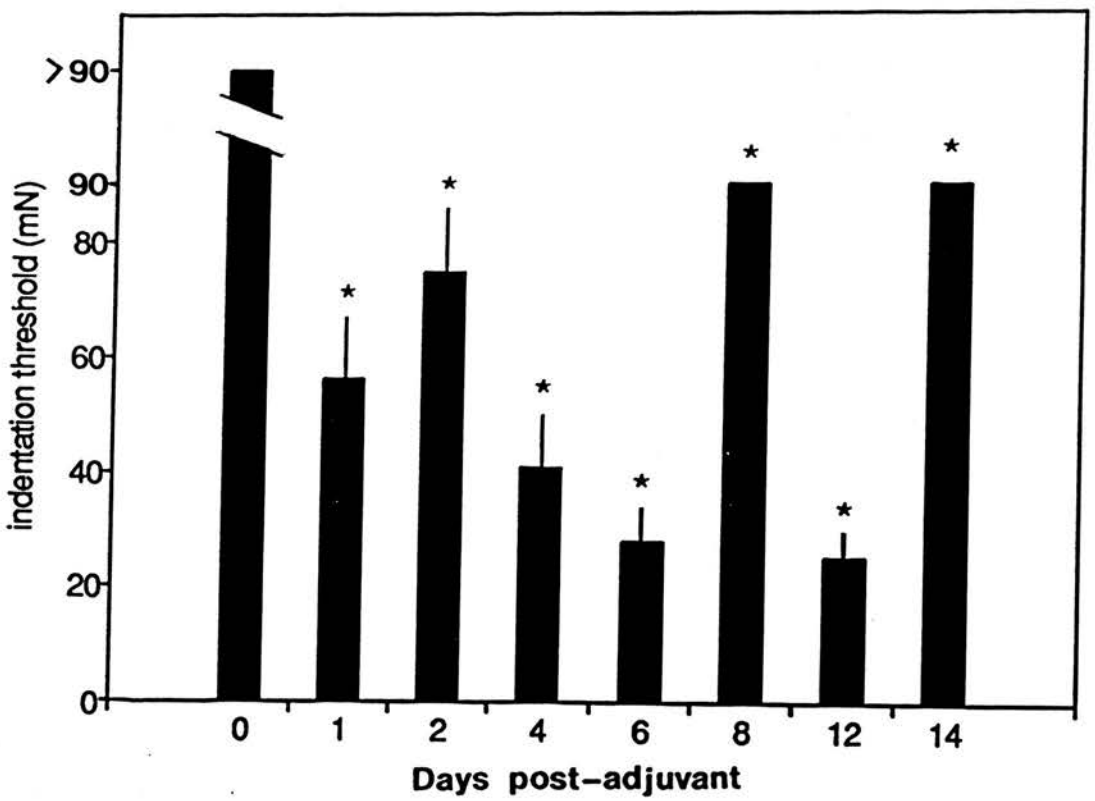


Fig 3.9 Mean values for withdrawal thresholds to mechanical stimuli using von Frey hairs for the left hindlimb of arthritic rats (n=4). Significantly lower mean thresholds than those for control rats are shown as * when $p < 0.05$ (Wilcoxon).

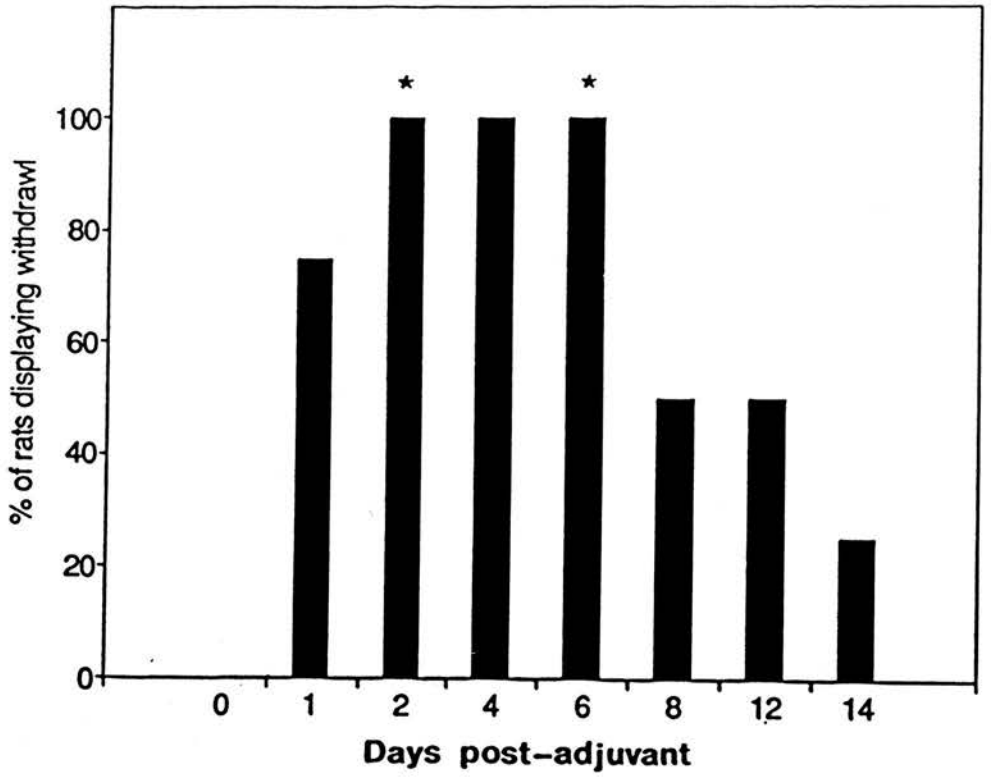


Fig 3.10 Percentage of arthritic rats displaying withdrawl to flexion and extension of the left ankle joint (n=4) over the 14 day period following injection of F.C.A.. Tests with a significantly greater numbers of rats displaying withdrawal when compared with controls are shown as * when $p < 0.05$ (Wilcoxon).

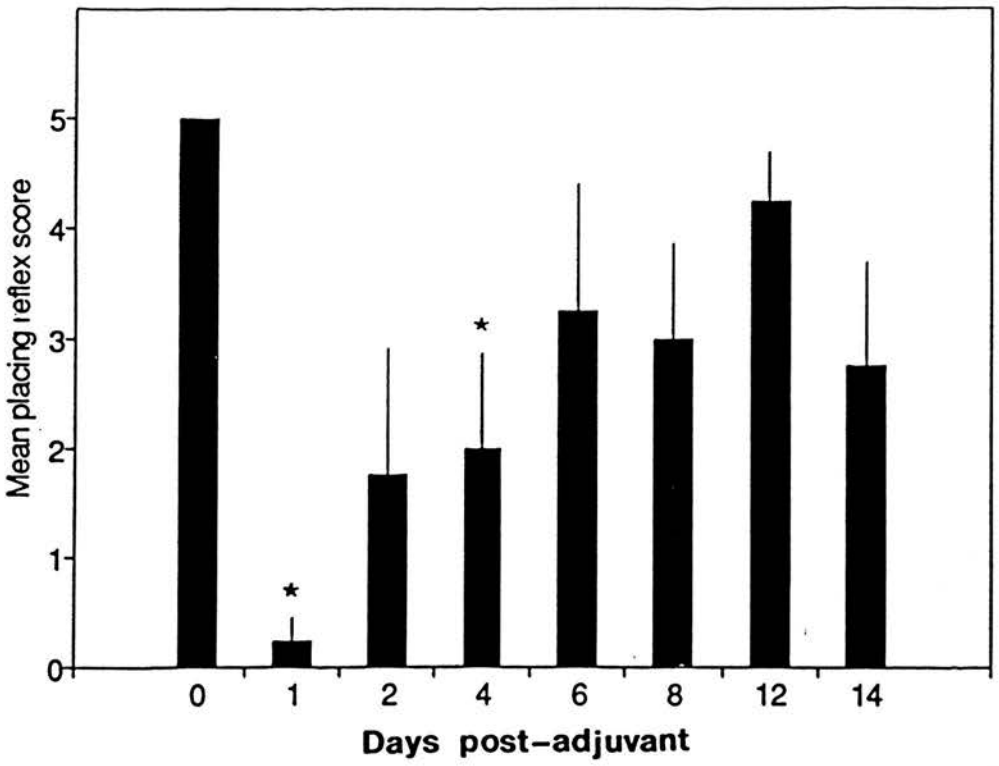


Fig 3.11 Mean placing reflex scores for the left hindlimb of rats injected with F.C.A. (n=4). Values significantly lower than those for control rats are shown as * when $p < 0.05$ (Wilcoxon).

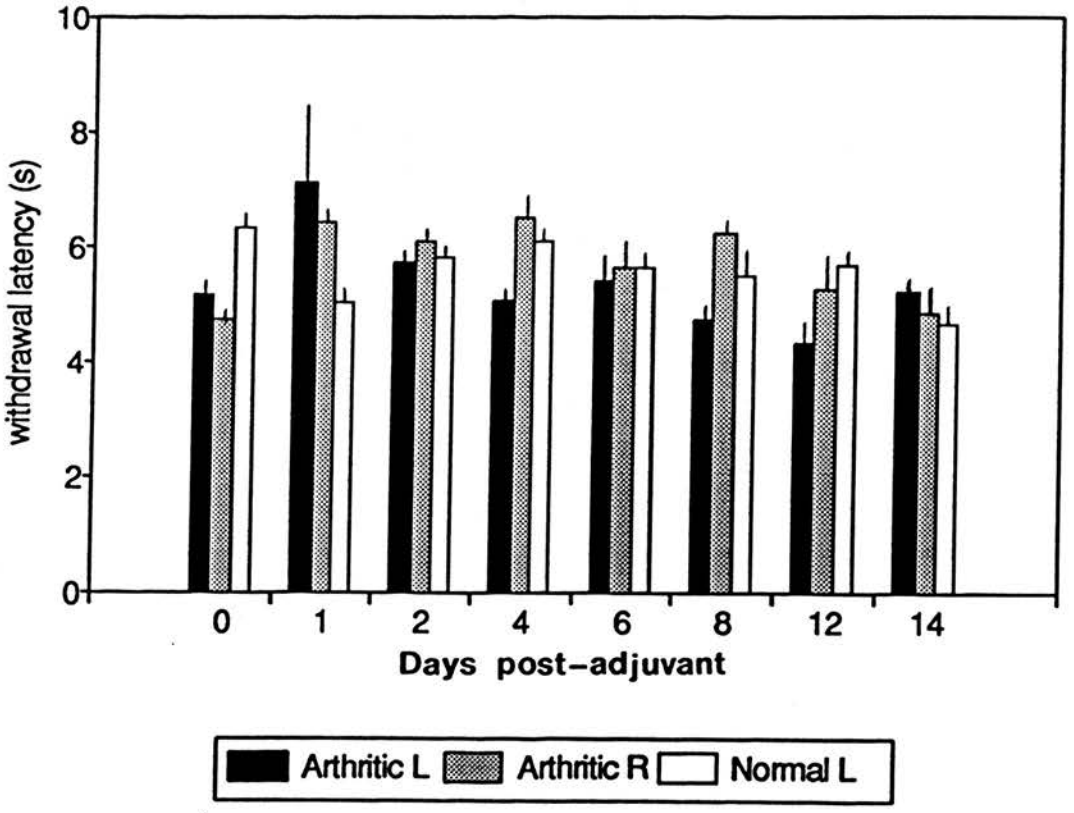


Fig 3.12 Mean withdrawl latencies to radiant heat stimuli for the 14 day period following injection of F.C.A. around the left ankle joint on day zero. Bars represent mean values for measurements from the injected left ankle (L, closed bars, n=4), contralateral non-injected ankle (R, shaded bars, n=4), and the left ankle (L, open bars, n=4) of control rats. The vertical lines above each bar represent the s.e.m..

Manipulation of the foot did not elicit noxious responses in either untreated controls or in the right limbs of arthritic animals. For the injected left ankle, the number of rats displaying withdrawl responses increased from from day one and remained elevated until day six. Thereafter there was a decline in the number of withdrawl responses elicited (fig. 3.10).

The average number of clear placing reflex responses was reduced a day after injection of adjuvant. This figure increased gradually up to day six when a steady level was reached (fig. 3.11). For uninjected limbs and untreated rats clear responses were obtained in all cases.

Withdrawal latencies for the radiant heat test were not significantly different between control and arthritic rats at any point following adjuvant injection (fig. 3.12).

3.2.4 Histopathology

The tibiotarsal joints of five normal rats, five rats at 15 days and five rats at 31 days post-injection were used for examining the histopathology of localized adjuvant arthritis.

Following the administration of adjuvant all rats developed a severe swelling around the ankle region of the injected left hindlimb. Following an initial vigorous swelling, a stable level was reached after about ten days. This level of inflammation and swelling was maintained throughout a thirty one day period of observation. Inflammation was restricted to the ankle joint region with little evidence of swelling in the paw or further up the leg. Contralateral limbs appeared to be unaffected by the treatment.

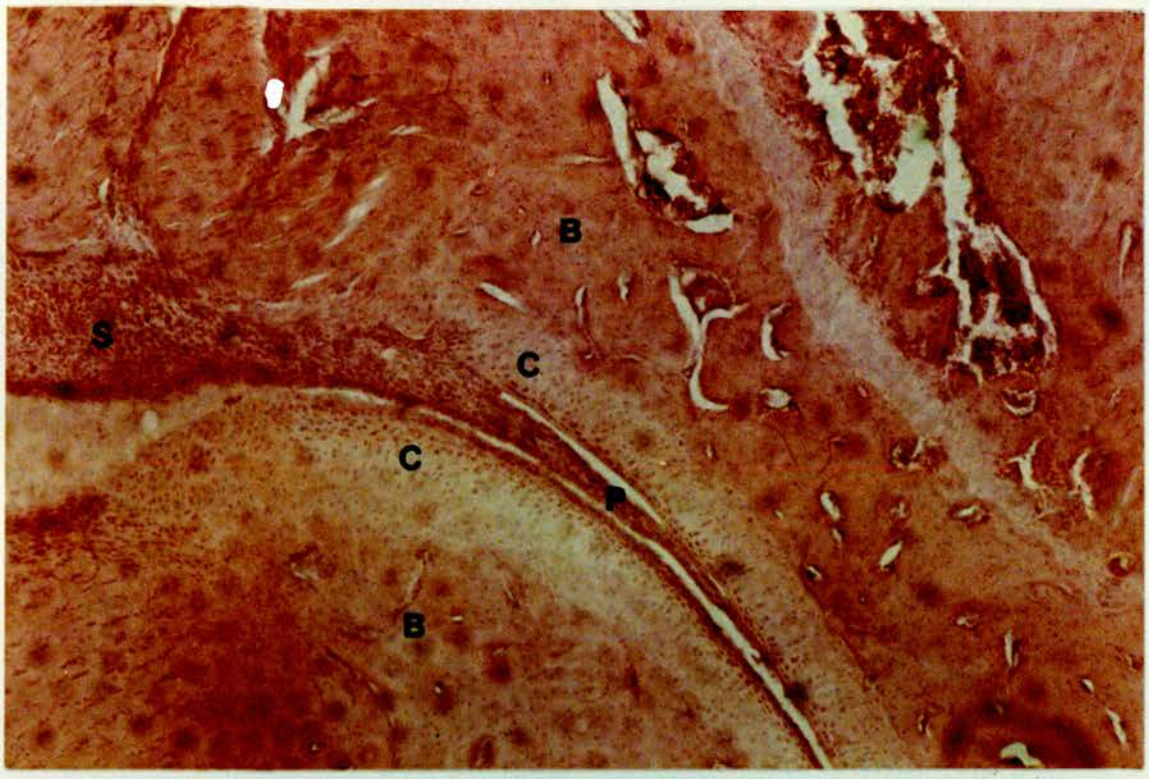
Histological examination of the ankle joints at fifteen days post-injection revealed the presence of a severe invasive periarthritis. The abundance of invading neutrophils and disintegration of the synovial lining were the most obvious changes seen in these joints. The joint cavity itself was relatively free from inflammation, although invasion of outer regions by fibrinoid synovial villi was prominent in all cases. Active fibrosis was also seen at sites where erosion was taking place around the margins of articular cartilage (figs. 3.13 - 3.15). Simultaneous osteoblastic production of new periosteal bone and osteoclastic erosion of existing bone was observed along some bone shafts (fig. 3.16).

Ankle joints from contralateral non-injected limbs had a normal histological appearance. Synovial tissues appeared to be healthy and no invasive cells could be seen in the surrounding connective tissue (figs. 3.17 - 3.18).

Examinations of bone sections taken from rats thirty one days after injection of adjuvant revealed that the arthritis had progressed to a more erosive condition. Osteoclast activity was evident at marginal cartilage and in subchondral bone (fig. 3.19 - 3.20). In the smaller tarsal bones of the paw severe erosion was seen in a small number of joints (fig. 3.23). Non-injected contralateral ankle joints from these rats were normal in appearance (figs. 3.21 - 3.22).

Fig 3.13 Micrograph (x10) of a 20μm thick transverse section through the tibio-tarsal joint of the arthritic left hind limb 15 days post-injection of F.C.A.. Marked cellular infiltration of and fibrin deposition in the synovium (S) is clealy present. Proliferation of the synovial tissue has led to the formation of an adhesive pannus (P) which has invaded the joint space. The initial stages of bone (B) erosion are occurring at the margins of the articular cartilage (C).

Fig 3.14 Micrograph (x10) of 20μm thick transverse section through the tibio-tarsal joint of the left hindlimb of an arthritic rat 15 days post-injection of F.C.A.. Severe periarticular invasion of neutrophils and breakdown of the synovial (S) lining are in evidence. Fibrin can be seen in the peripheral margins of the joint space (JS). Adhesion of proliferative synovial tissue and bone erosion are present at the marginal cartilage (MC).


B
S
C
C
P
B

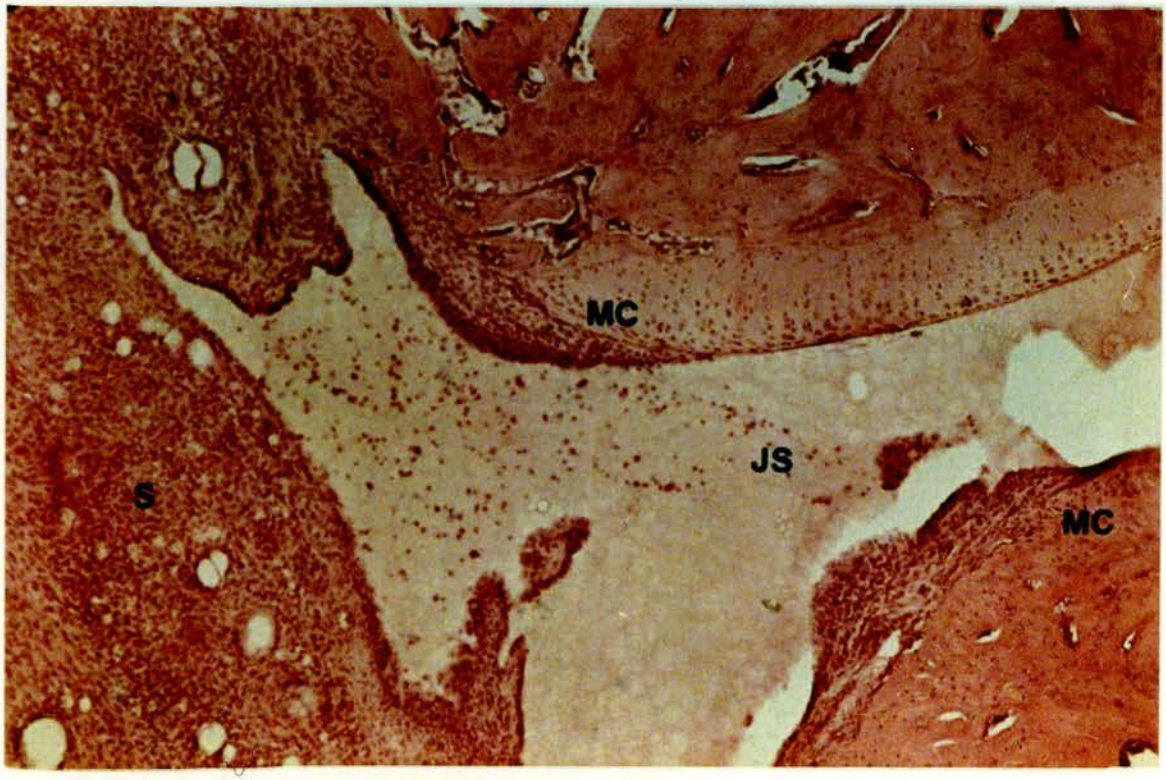
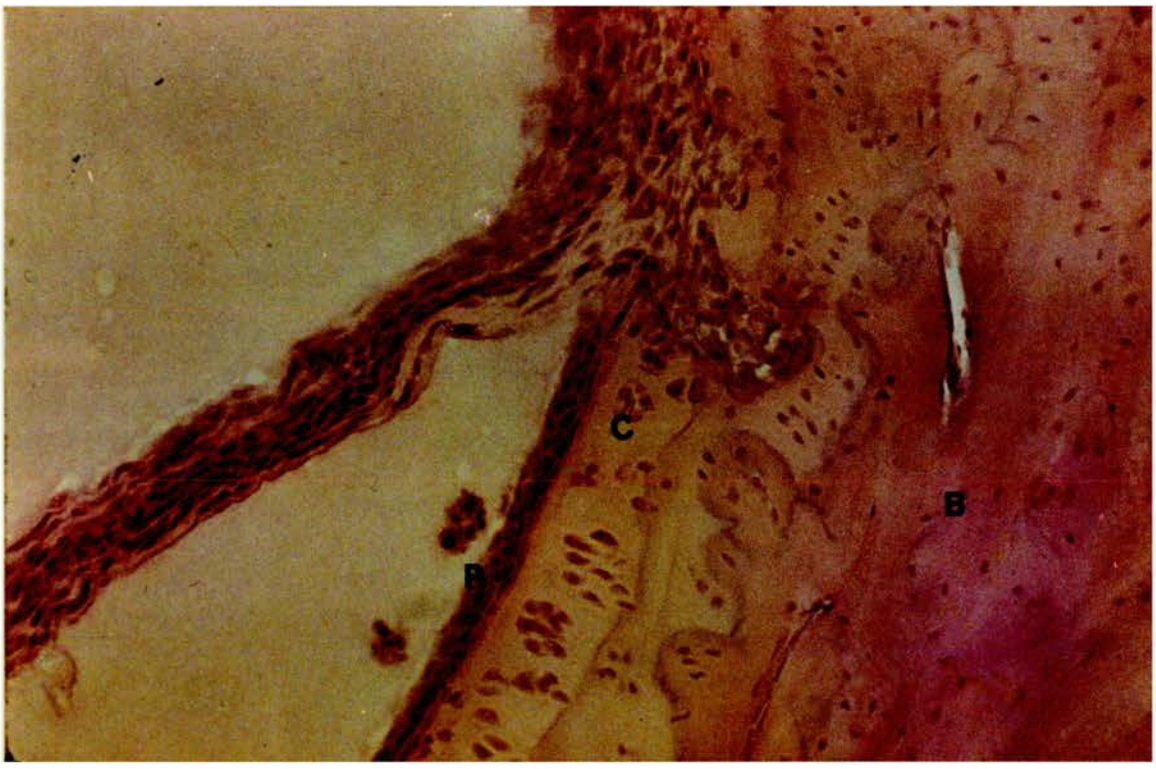

MC
JS
S
MC

Fig 3.15 Micrograph (x40) of a 20μm thick transverse section through the left tibio-tarsal joint of an arthritic rat 15 days post-injection showing detail of the marginal cartilage. Adhesion of pannus (P) can be seen on the surface of the joint cartilage (C). Marginal cartilage breakdown is occurring beneath the adhesive layer. Subchondral bone is also labeled (B).

Fig 3.16 Micrograph (x20) of a 20μm thick transverse section through the distal shaft of the tibia from the left hindlimb of an arthritic rat 15 days post-injection of F.C.A.. Osteoclastic erosion (O of existing bone and simultaneous osteoblastic production of new bone occurring along the shaft of the tibia (T). The connective tissue (CT) contains large numbers of invasive cells.


C
B
B

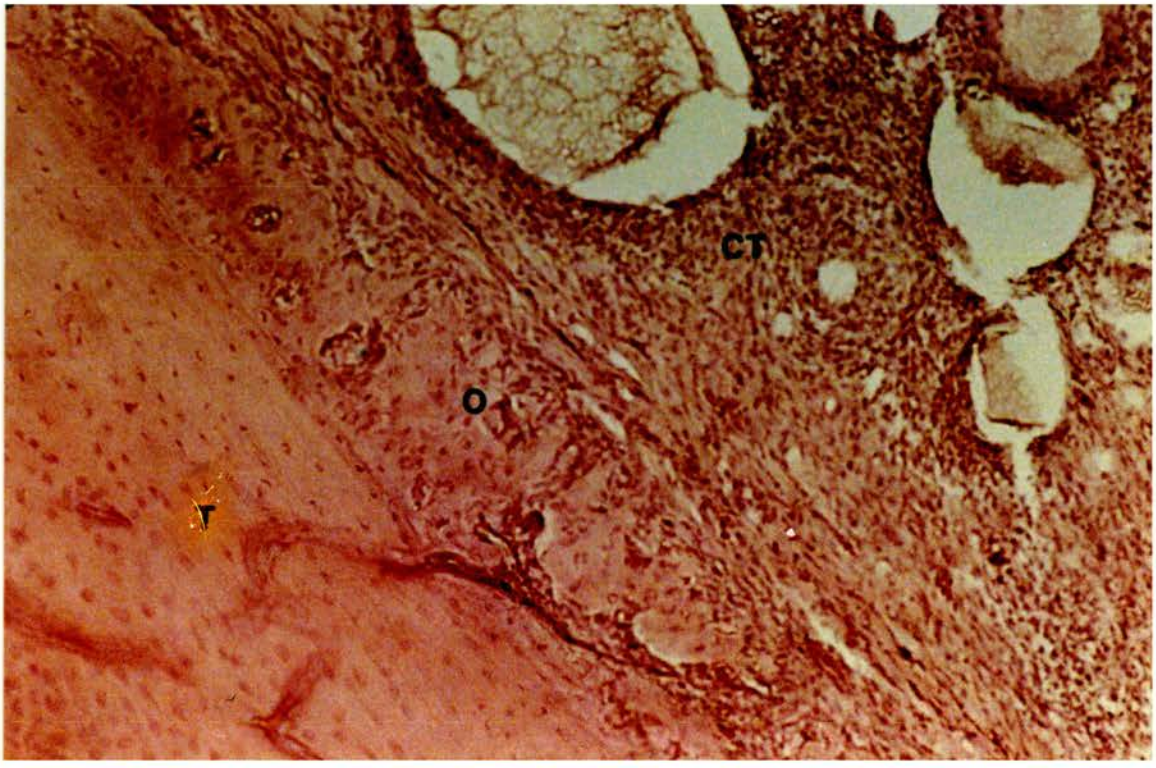

CT
O
T

Fig 3.17 Micrograph (x20) of a 20μm thick transverse section through the uninjected right tibio-tarsal joint of an arthritic rat 15 days post-injection of F.C.A.. The synovium (S) and synovial lining cells (SL) are healthy in appearance, and clear from any fibrin or invasive cells. The marginal zones of cartilage are intact and joint space (JS) is totally clear.

Fig 3.18 Micrograph (x10) of a 20μm thick transverse section through the uninjected right tibio-tarsal joint of an arthritic rat 15 days post-injection of F.C.A.. The synovial tissue (S) is normal in appearance and free from invasive cells. Apposing cartilage and underlying bone are healthy in appearance.

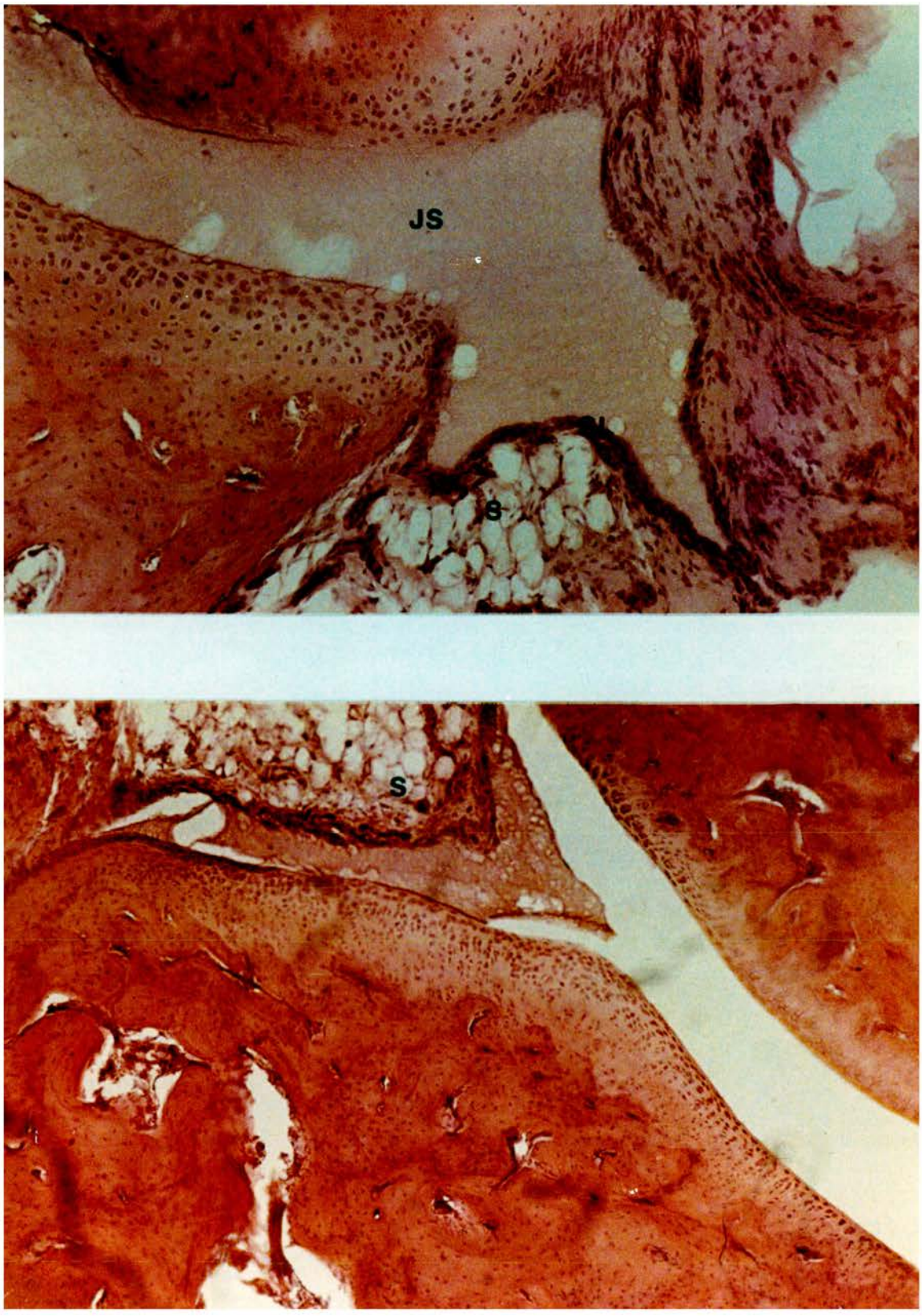
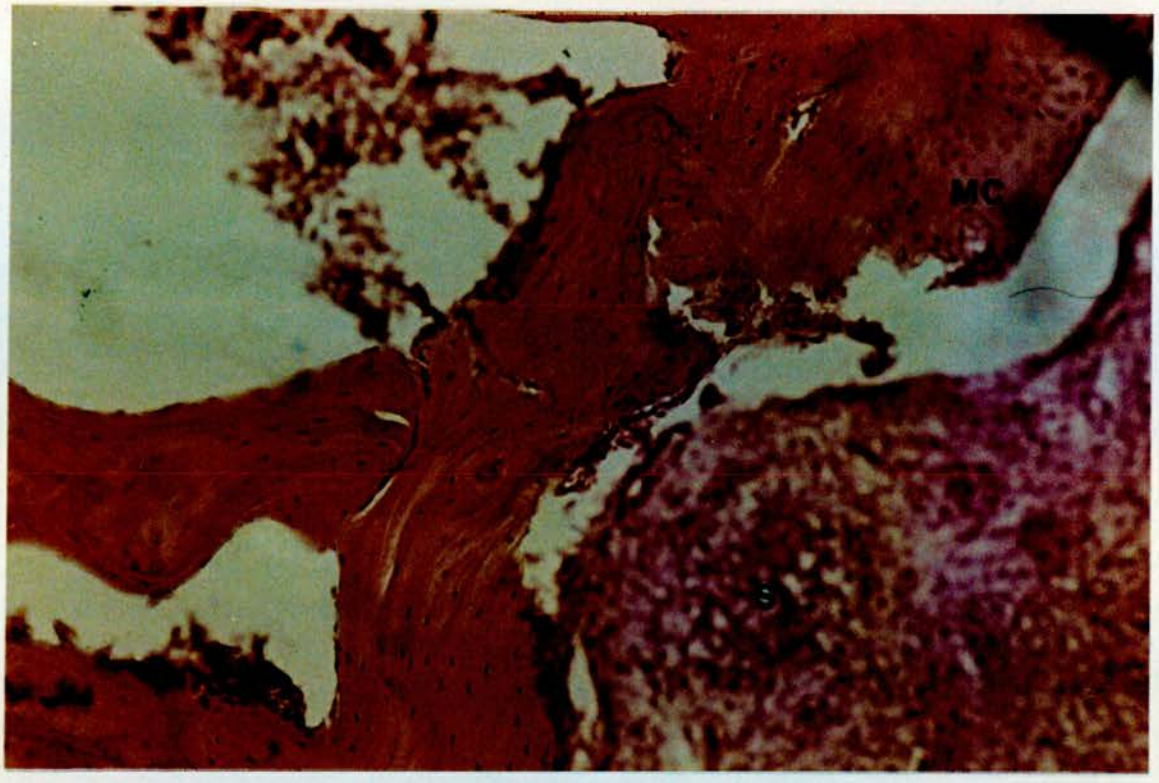

JS
S
S

Fig 3.19 Micrograph (x40) of a 20μm thick transverse section through the arthritic left tibio-tarsal joint 31 days post-injection of F.C.A., showing detail of the marginal cartilage. Severe cellular infiltration of synovium (S), marginal erosion of articular cartilage (MC), and evidence of osteoclast activity in subchondral bone.

Fig 3.20 Micrograph (x10) of a 20μm thick transverse section through an arthritic left tibio-tarsal joint 31 days post-injection of F.C.A.. The synovium (S) contains large numbers of invasive cells, and a fibrinoid synovial villus can be seen at the margin of the joint space . Marginal erosion of cartilage can be seen, but the apposing cartilage (C) is otherwise quite healthy in appearance.


MC

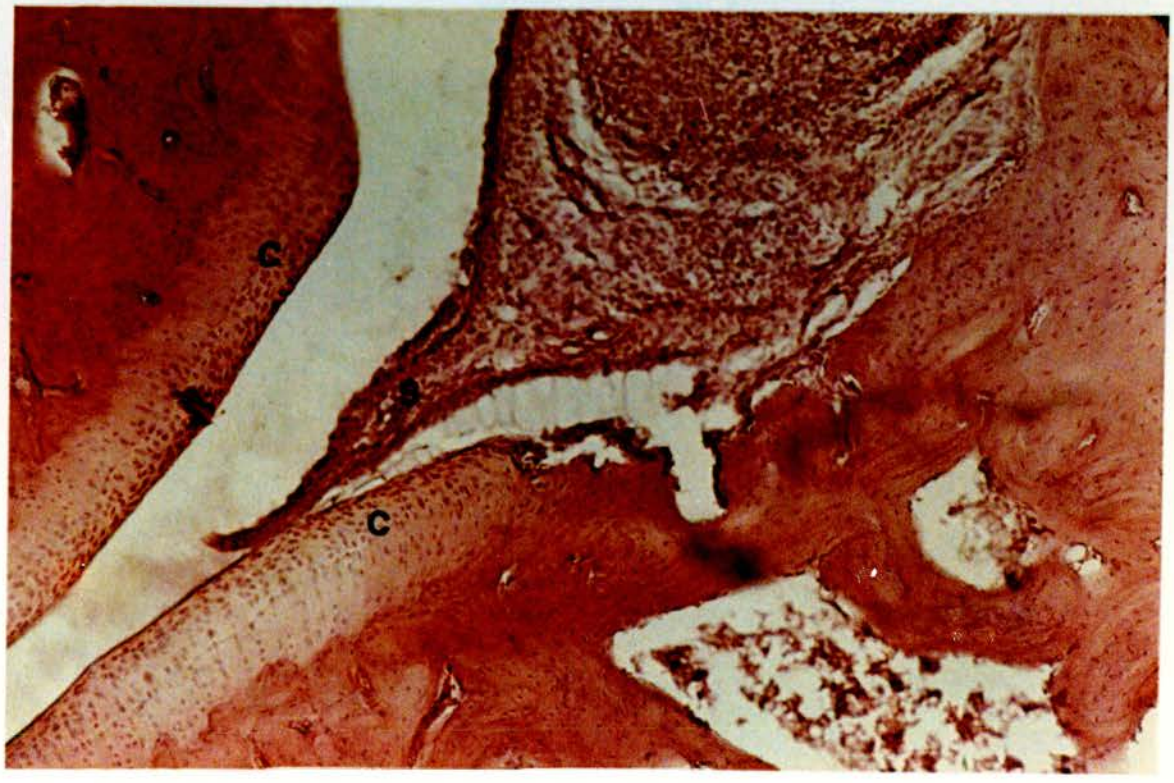
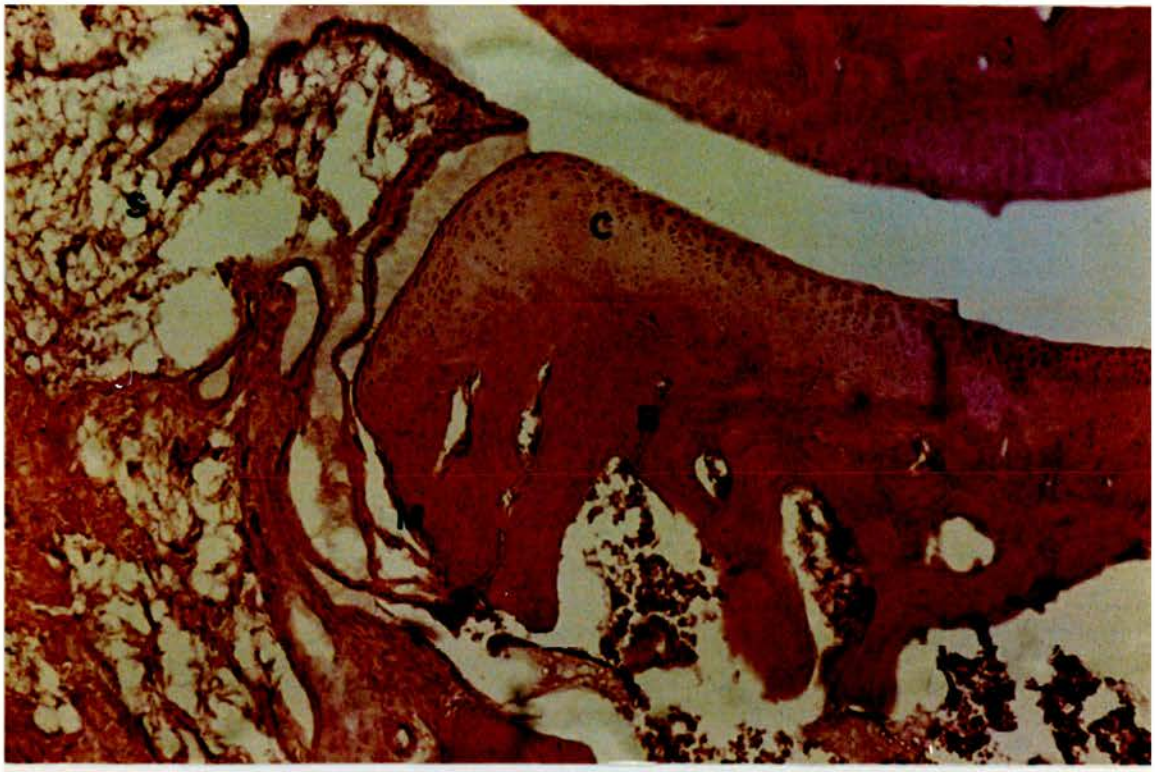

C
C

Fig 3.21 Micrograph (x10) of a 20μm thick transverse section through the uninjected right tibio-tarsal joint 31 days post-injection of F.C.A. The synovium (S), synovial lining cells and marginal zones (M) of attachment are normal in appearance and free from fibrin or invasive cells. The apposing articular cartilage (C) and underlying bone (B) are also normal and healthy.

Fig 3.22 Micrograph (x20) of a 20μm thick transverse section through the noninjected right tibio-tarsal joint 31 days post-injection of F.C.A.. Normal synovial lining and synovium (S). Articular cartilage (C) is normal and healthy in appearance.


S
C
M

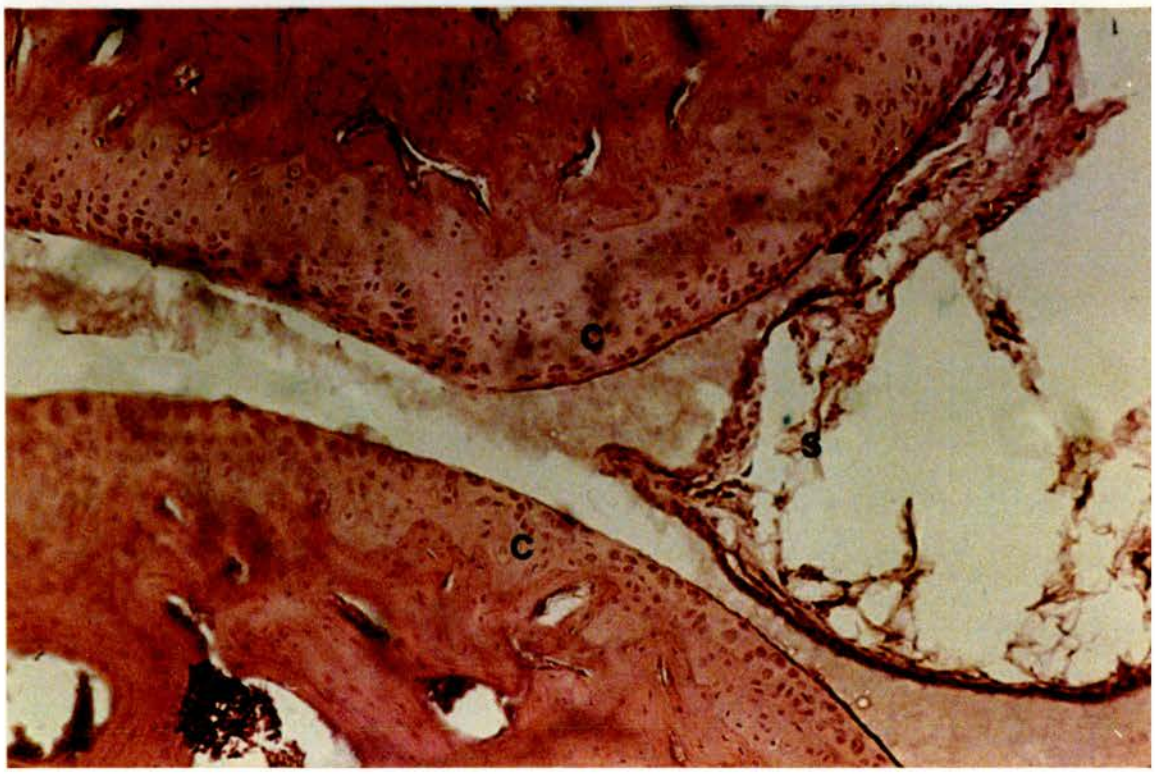
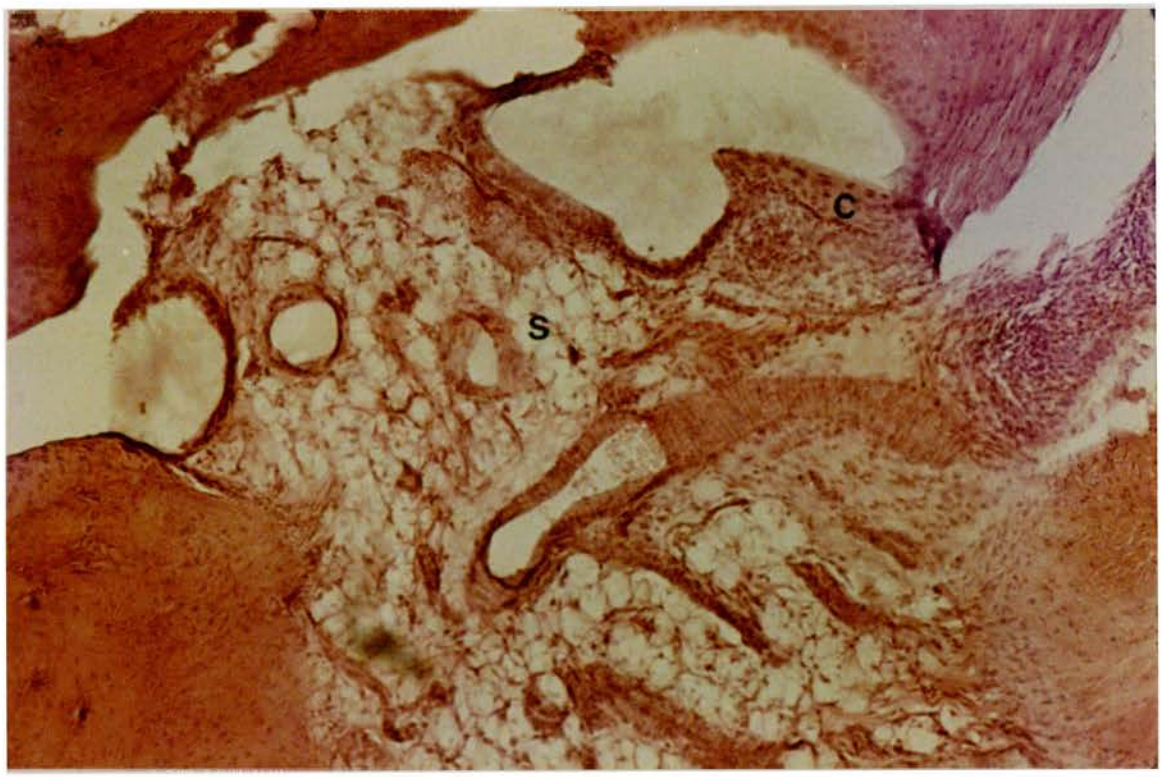

C
S
C

Fig 3.23 Micrograph (x10) of a 20μm thick transverse section through the arthritic left hind paw 31 days post-injection of F.C.A, showing detail of the small tarsal bones. Complete disintegration of synovium (S) and erosion of existing bone has occurred, leaving unsupported articular cartilage (C).


C
S

3.3 DISCUSSION

3.3.1 Electrophysiology - in vivo

The high-threshold slowly adapting mechanoreceptors described above had response characteristics comparable to those of previously identified articular nociceptors in the rat ankle joint (Guilbaud et al., 1985). Afferent fibre conduction velocities were in the A-delta or C fibre range, and fatigue developed following repeated mechanical stimuli. Afferent units had either a low level of backround discharge or had none at all. Nociceptors from the cat knee joint have also been reported to have similar characteristics, responding mainly to intense mechanical stimuli, to extreme flexion or extension, or to rotations of the joint. (Schaible & Schmidt, 1983a,b). These units in the cat had either a low background discharge or were silent.

Slowly adapting mechanoreceptors with receptive fields in the tissues of the arthritic rat ankle joint had lower activation thresholds and higher levels of ongoing activity than those from normal joints. These results are in agreement with those obtained by Guilbaud et al. (1985) from the ankle joints of rats with adjuvant-induced polyarthritis. Similar findings have also been reported for cat knee joints acutely inflamed using intra-articular injection of kaolin and carrageenan (Coggeshall et al., 1983). In the acutely inflamed cat knee joint articular receptors with A-delta and C fibre afferents had a heightened sensitivity to joint movements, and a previously absent background discharge (Coggeshall et al. 1983; Grigg et al., 1986; Schaible & Schmidt, 1985; 1988a).

In both the present study and that of Guilbaud et al. (1985) a larger number of receptors could be identified in arthritic tissues by probing mechanically on the joint surface, with individual receptive fields corresponding to single afferent units. This finding is in contrast to the situation in the cat where multiple receptive fields for single units are quite commonly found in the inflamed joint (Schaible & Schmidt, 1985).

Direct comparison of receptor characteristics both before and after the development of an acute inflammation was achieved in the cat knee joint (Schaible & Schmidt, 1988a). Inflammation led to sensitization of C and A-delta high-threshold units and induction of mechanosensitivity in units which were unresponsive to mechanical stimuli in the normal joint. Resting activity consisting of irregular discharges was also induced.

More recently studies on the effects of inflammation on sensory receptors from other tissues have been reported. Sensitization of C fibre polymodal nociceptors to both mechanical and thermal stimuli has been described following induction of carrageenan inflammation in the skin of the rat (Kocher et al., 1987). Only those units developing ongoing activity were sensitized by the inflammation. Cutaneous C-polymodal nociceptors in the rabbit ear have been shown to develop background discharge following ultraviolet irradiation sufficient to produce evidence of delayed inflammation (Szolcsanyi, 1987). In the muscle of the cat, carrageenan-induced inflammation sensitized C and A-delta nociceptors, and caused increases in background activity (Berberich et al., 1988). There was no apparent correlation between the mechanical threshold of a receptor and its level of background activity

in inflamed muscle. Finally, in studies on the urinary bladder, mustard oil-induced acute inflammation caused the development of ongoing activity and sensitization to bladder distension for receptors with unmyelinated afferent axons (Habler et al., 1990). These receptors were previously insensitive to mechanical stimuli.

The results described above suggest that the sensitization of C and A-delta nociceptors is probably crucial for the development of inflammatory pain and hyperalgesia. During inflammation the recruitment of receptors which in normal tissues are non-responsive to mechanical stimuli will lead to an enhancement of nociceptive input to the spinal cord and higher perceptual centres. A number of different terms have been used to describe this type of receptor which becomes sensitive to perturbation following tissue damage. Schmidt and colleagues have used the term 'sleeping nociceptor', and along similar lines Handwerker and his colleagues have called them 'silent nociceptors' (personal communication). These descriptors do not, however, attempt to ascribe a biological function to these sensory elements. Perhaps the best way to describe them is as 'inflammation receptors', a term first used by Iggo (1988), to identify their key involvement in inflammation.

From a review of the present literature it would seem that the changes in receptor characteristics seen in acutely inflamed tissues are superficially the same as those observed in the chronic inflammation of adjuvant polyarthritis and localized adjuvant arthritis. It seems likely that alterations in nociceptor function, occurring as a result of chronic inflammation, may only be revealed using pharmacological techniques, and as such investigations of this type are of great interest.

3.3.2 Electrophysiology - in vitro

Articular sensory receptor populations identified *in vitro* were similar to those found in rat ankle joints from *in vivo* electrophysiological studies. Furthermore, receptive fields, mechanical activation thresholds, and response characteristics to mechanical stimuli did not differ from those seen *in vivo*. Time-related changes in receptor behaviour did not occur in these experiments, and no obvious soft tissue pathology was observed for periods of up to eight hours after removal of the limb. The long-term maintenance of receptor responsiveness *in vitro* indicates that any biochemical factors essential for normal receptor excitability have not been washed out of the receptor environment.

Articular sensory receptors from arthritic joints studied *in vitro* displayed essentially the same characteristics as those seen *in vivo*. Thus, compared to the situation in normal joints, arthritic joints had an increased number of mechanosensitive receptive fields. These receptors had a lower mean mechanical activation threshold, and a higher level of spontaneous resting discharge than those found in normal joints. The similarity between the situation found here *in vitro* and that seen *in vivo* suggests that the heightened sensitivity of these receptors in arthritic joints is not dependent on the continued presence of blood borne components, or on influences from the central nervous system. These results support the idea that an *in vitro* joint preparation could be used to examine the effects of chronic inflammation on the sensitivity of articular sensory receptors. The use of a unilateral arthritis provides the opportunity to examine both the arthritic and non-arthritic, contralateral joints from the same animal.

3.3.3 Behaviour and gross pathology

The time course of development of localized adjuvant-induced arthritis compares well with that seen in polyarthritis. An initial swelling at the site of injection was followed by a secondary inflammation of the ankle joint which persisted for the duration of the experiment. The major difference seen in the present condition is its highly localized nature, and lack of whole body effects. The localized nature of the disease is most likely to be a result of the comparatively low dose of adjuvant used (Ward & Jones, 1962) and the subdermal site of injection around the ankle joint (see Billingham, 1983). In a similar model of localized adjuvant-induced arthritis, injection of the same dose of adjuvant into the foot pad produced contralateral inflammation after a period of fourteen to thirty six days in 10% of rats (Stein et al., 1988). Results from recent studies using adjuvant-induced inflammation of one hindpaw (Stein et al., 1988) compare well with those described in the present investigation. Patterns of weight gain, tissue swelling and development of hyperalgesia all follow a similar time course in both studies.

Tests for hyperalgesia have been performed extensively in rats with adjuvant polyarthritis (see Rosenthale & Capetola, 1982). Responses indicative of pain such as vocalization and struggling can readily be elicited in polyarthritic rats by a variety of stimuli including direct pressure on the paw as in the Randall-Selitto test (Butler et al., 1985; Calvino & le Bars, 1986; Hara et al., 1984; Hirose & Jyoyama, 1975; Kayser & Guilbaud, 1981; Kayser & Guilbaud, 1983; Winter et al., 1979) or flexion of the ankle joint (Capetola et al., 1978;1980; Kuzuna &

Kawai, 1975).

In the present study the use of these tests revealed a rapid onset of hyperalgesia in the treated paw which was maintained for the two weeks post injection over which measurements were carried out. A reduction in withdrawal threshold to pressure produced by a graded series of von Frey hairs also demonstrated development of hyperalgesia in the injected paw. Reduction in nociceptive thresholds were restricted to the injected limb for flexion-extension and von Frey hair tests. However, a reduction in the withdrawal threshold for application of gradually increasing pressure was seen for both inflamed and non-inflamed joints. It is most likely that this effect was produced as a result of rats associating the test with a painful experience. Avoidance behaviour of this type is also evident in the responses of uninjected control rats where withdrawal thresholds were also significantly reduced after a delayed period.

The most obvious effect of joint inflammation was a reluctance to place any great weight on the paw of the inflamed ankle. In many cases an obvious limp was present for short periods, with animals displaying distinct guarding behaviour. The subjective measure of weight bearing scores used here shows that a clear reduction in the use of the inflamed limb for walking and standing occurs throughout the test period. These results compare well with those obtained in similar studies where other forms of inflammation affecting only one limb were examined (Coderre & Wall, 1987; Okuda et al., 1984; Otsuki et al., 1986). This type of test has an advantage over measurements using a force transducer (Van Arman et al, 1970; Otsuki et al., 1984) as no prior training is required, and stress caused by restraint of the animal is considerably reduced (Coderre & Wall, 1987). Examination of placing reflexes also indicated a

reluctance of the animals to move their inflamed joint. Results from these tests suggest that animals with chronically inflamed ankle joints experience a sustained level of discomfort which restricts their use of the affected limb, however, this probably also reflect the animals reluctance to experience acute pain when the joint is moved or allowed to participate in any load bearing.

The finding that withdrawal latencies to noxious heat stimuli were not significantly different between treated and non-treated paws or control animals suggests that lowered thresholds to mechanical stimuli are not accompanied by reductions in thresholds to thermal stimuli. However, it is possible that the test was not sensitive enough to detect any changes in threshold that may have occured as a result of tissue inflammation.

Weight loss and retardation of growth were seen during the initial stages of inflammation. Although less severe, this pattern is similar to that reported for studies on rats with polyarthritis (Mathur et al., 1977; Newbold, 1969; Walz, 1971). The reasons for retarded weight gain in arthritic rats are not entirely clear. It has been suggested that reduced motor activity related to the severity of pain is responsible for a reduction in feeding and thus weight loss (Chudler & Dong, 1983; Morton & Griffiths, 1985). However, in studies where food was made available so that no locomotion was required for the animals to consume it, the same pattern of weight loss was seen (Colpaert & Van den Hoogen, 1983). Other factors may be involved in reduced eating and weight loss, including disease- and stress-related loss of appetite (Morton & Griffiths, 1985).

3.3.4 Histopathology

Examination of sections of tibiotarsal joint from rats with localized adjuvant-induced arthritis revealed the presence of a chronic invasive periarthritis combined with a proliferative synovitis. In comparison with adjuvant-induced polyarthritis (Pearson & Wood, 1959), the present condition is substantially less severe in terms of bone erosion, and very little degradation of marginal and sub-chondral bone occurs. Bony ankylosis, a common feature of polyarthritis, is rarely seen in this disease. The most important difference seen in the present model is its localized nature, with the contralateral, non-injected tibio tarsal joint having a normal histological appearance.

As in adjuvant polyarthritis the fibrin-like material seen early on in the disease at the margins of the articular cavity probably comes from the exudation of blood (Pearson & Wood, 1963; Mohr & Wild, 1976). This material appears to induce rapid proliferation of the synovium (Pearson & Wood, 1963; Mohr & Wild, 1976). The presence of invasive cells around the synovium, tendon sheaths, bursae and periosteal surfaces by inflammatory cells seen at day fifteen represent signs of the local inflammatory process. Identification of different cell types is difficult using the present staining techniques. However, at this stage of polyarthritis various cell types have been identified around the synovium including mast cells (Van Arman, 1976; Mohr & Wild, 1976) polymorphs, monocytes and lymphocytes (Pearson & Wood, 1963; Nusbickel & Troyer, 1976).

A prominent feature of adjuvant polyarthritis, and indeed human rheumatoid arthritis, is the presence of a granulomatous outgrowth of

tissue from the synovium known as pannus (Pearson & Wood, 1963; Mohr & Wild, 1976). This characteristic of the chronic arthritic lesion is also seen in the the present condition where invasion of the joint cavity by fibrinoid synovial villi is clearly present by day fifteen. As in polyarthritis, erosion of marginal cartilage can be seen at day fifteen and to a greater extent at day thirty one where contact is made with fibrinoid synovial villi.

Histological examination of the tibio-tarsal joint in localized adjuvant arthritis has revealed a pathology that can probably be described most accurately as a chronic proliferative synovitis. Although the appearance of the periarticular tissues are similar to that seen in adjuvant polyarthritis, the limited nature of the bone and cartilage destruction seen in this model differs conspicuously from the severe erosion prevalent in established polyarthritis. In this aspect, the present model bears a closer resemblance to rheumatiod arthritis in humans.

3.3.5 Further points for discussion

Levels of neuropeptides involved in nociceptive processes, such as substance P, CGRP and the opioid peptides, have been shown to be increased bilaterally in the dorsal horn of the spinal cord and in primary afferent neurones of rats with adjuvant polyarthritis (Lembeck et al., 1981; Chery-Croze et al., 1985; Levine et al., 1985; Millan, 1986; Oku et al., 1987). These changes are thought to occur at least partly as a result of a sustained high level of nociceptive input. Similar increases have recently been shown to occur unilaterally in

localized arthritis (Stein et al., 1987, 1988; Nahin et al., 1990; Smith et al., 1990) providing further evidence that this represents a model of chronic inflammatory pain.

3.4 CONCLUSIONS

The results obtained in this study suggest that localized adjuvant-induced arthritis represents a suitable model for the study of pain associated with chronic inflammation. A large number of characteristics associated with alterations in nociceptive systems in adjuvant polyarthritis have also been demonstrated in the present model. Furthermore, that many of the characteristics described above have been shown to be present only on the side ipsilateral to the inflammation, provides a within animal experimental control, which is not possible in adjuvant polyarthritis. It is concluded, therefore, that localized adjuvant-induced arthritis has considerable scientific and ethical advantages over adjuvant polyarthritis for the neuropharmacological study of arthritic pain.

SECTION IV

EFFECTS OF CAPSAICIN ON ARTICULAR SENSORY RECEPTORS IN THE RAT ANKLE JOINT

SECTION IV

EFFECTS OF CAPSAICIN ON ARTICULAR SENSORY RECEPTORS IN THE RAT ANKLE JOINT

4.1 INTRODUCTION

Cutaneous application of capsaicin, the pungent ingredient of hot pepper, causes burning pain and flare that spreads beyond the area of contact (Bernstein et al., 1981; Carpenter & Lynn, 1981). Similar excitatory actions occur in the airways and in many visceral organs. Excitation of cutaneous C-polymodal nociceptors is evoked in all mammalian species tested, including cat (Foster & Ramage, 1981), rabbit (Szolcsanyi, 1987), rat (Kenins, 1982; Szolcsanyi et al., 1988) and man (Konietzny & Hensel, 1983). Other types of C fibre receptor in the skin, including high threshold and low threshold mechanoreceptors are reported not to be excited (Foster & Ramage, 1981; Kenins, 1982; Szolcsanyi, 1988). Cutaneous sensory receptors with A-fibre afferents are generally unaffected by capsaicin, although a small population of A-delta nociceptors in the rat hairy skin are excited (Szolcsanyi et al., 1988).

The effects of capsaicin have also been examined on axons in nerve trunks and on neuronal cell bodies. Capsaicin causes depolarization of the rat isolated sciatic (Hayes et al., 1984) and vagus (Marsh et al., 1987) nerves, and somata from rat nodose (Marsh et al., 1987) and dorsal root (Baccaglini & Hogan, 1983; Heyman & Rang, 1985) ganglia.

Depolarization is only seen in a sub-population of C-type ganglion cells, suggesting that capsaicin does not affect all C fibre afferents (Heyman & Rang, 1985; Marsh et al., 1987).

In rat sensory neurones in culture, capsaicin opens cation-selective channels, which can be observed using patch-clamp recording (Bevan & Forbes, 1988). The channels are activated in isolated membrane patches suggesting that they are directly gated by the capsaicin, and no second messengers are involved. These capsaicin-gated channels appear to differ from channels described previously in dorsal root ganglion cell membranes, having high permeability both to sodium and calcium ions, and unusual rectifying properties.

Examination of the effects of drugs on sensory receptors using intra-arterial injection relies on access to receptor terminals via the local micro vasculature. Thus, if a sensory receptor is not excited, it is difficult to tell if this is due to the drug being ineffective on that particular unit, or if there is a problem of access. In order to test the resposiveness of sensory receptors to drugs injected intra-arterially a chemical known to produce receptor excitation must be used. Injection of K^+ has previously been used by other workers. However, the excitatory effect is not selective for receptors with unmyelinated or finely myelinated afferent fibres. In the present investigation the effects of capsaicin on articular sensory receptors were examined to determine whether the drug could be used as a selective pharmacological probe for the excitation of nociceptive afferents. Experiments were conducted using normal and arthritic rats, with effects being examined both <u>in vivo</u> and <u>in vitro</u>.

4.2 RESULTS

The effects of capsaicin were examined on two types of afferent unit: high-threshold slowly adapting mechanoreceptors with receptive fields in the joint capsule, and 'chemosensitive' units which were excited by capsaicin and other chemicals (i.e. 5-HT, bradykinin or prostanoid agonists) but for which no receptive fields for mechanical stimuli were found (table 4.1). Injections of capsaicin were carried out at the end of experiments in which the effects of other drugs were investigated.

4.2.1 In vivo electrophysiology

4.2.1.1 Excitatory effects on mechanoreceptors

Normal joints

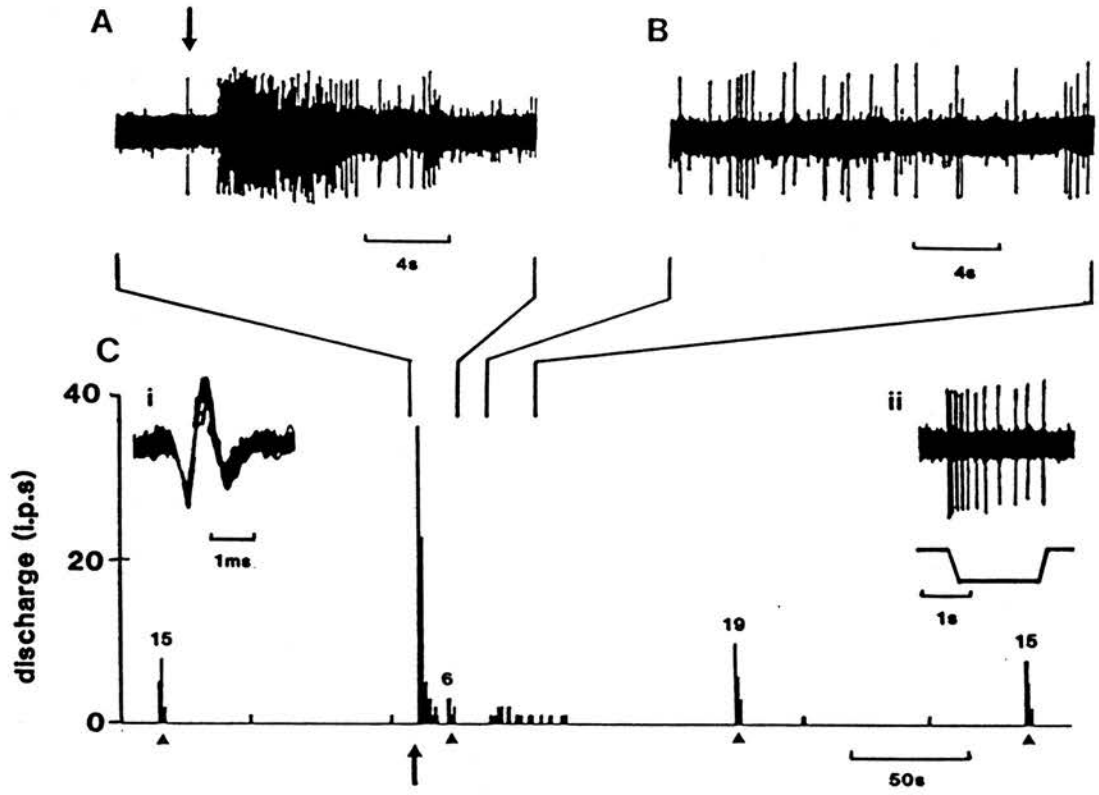
From nine experiments, thirteen high-threshold slowly adapting mechanoreceptors with a mean afferent fibre conduction velocity of 4.4 ± 1.8 ms^{-1} (range: 0.5 - 16.6 ms^{-1}) were examined. Since the majority of these units had previously been exposed to other excitatory substances, they had an enhanced level of ongoing activity of 0.6 ± 0.1 i.p.s.

Capsaicin (1 - 10 μg, i.a.) consistently activated all of the units tested (table 4.1). An example of the excitatory effect of capsaicin is illustrated in figure 4.1. In twelve (92%) units injection of capsaicin evoked responses of short latency (3 ± 0.7 s), short duration (14.3 ± 2.6 s) and high discharge frequency. The mean peak discharge, obtained over a 15 s time period, was 3 ± 0.4 i.p.s., and the mean total number

Table 4.1 Summary of the number of mechanonociceptors and chemosensitive units responding to capsaicin.

	UNITS	n	no. of units displaying each type of response	
			rapid	delayed
NORMALS	mechanoreceptors	13	12 (92%)	10 (77%)
	chemosensitive	4	4 (100%)	3 (75%)
ARTHRITIC	mechanoreceptors	11	11 (100%)	7 (64%)
	chemosensitive	6	6 (100%)	4 (67%)

Fig. 4.1 Response of a slowly adapting articular mechanoreceptor (c.v. = 0.8 ms^{-1}) from an arthritic rat to the injection of 5 μg capsaicin. (A) Neurogram showing the firing pattern for rapid excitation induced by capsaicin. The time of injection of 5μg capsaicin (i.a.) is indicated by the arrow. (B) Neurogram showing the firing pattern for delayed excitation caused by capsaicin. (C) Computer-generated plot illustrating the response of the mechanoreceptor unit to the same injection of capsaicin (given at arrow) shown in the neurograms. Each collection bin (bar) represents a 1 s time period. The time periods corresponding to the neurogram traces are indicated above the graph. Responses to mechanical stimuli (applied at arrow heads) are also shown, with the number of evoked action potentials per stimulus given above each response. The inset traces show (i) 30 superimposed triggered oscilloscope sweeps of the recorded unit action potential, and (ii) the slowly adapting response of the unit to the standard mechanical stimulus. This unit responded with both rapid and delayed excitation. Mechanical responsiveness was first reduced (40% of control) following the initial excitation, and was then enhanced (127% of control) on the next stimulus.


A
B
4s
4s
C
40
20
0
discharge (i.p.s)
i
1ms
ii
1s
15
6
19
15
50s

of evoked action potentials above basal discharge (delta Σx) was 35 ± 6. Unit responses to a standard dose of 10 μg are shown in figure 4.2. Repeated injections of 1 - 10 μg doses at intervals of 15 minutes evoked a consistent response. Application at shorter intervals caused a reduction of the response to develop (fig 4.3). Dose-dependent effects were observed in four units.

In addition to rapid excitation, capsaicin also evoked a delayed (47 ± 6.3 s), longer lasting (79 ± 10 s) increase in activity in ten (77%) units (fig. 4.1). The mean peak discharge, obtained over a 15 s time interval, was 1.8 ± 0.4 i.p.s., and the mean total number of action potentials evoked above basal discharge (delta Σx) was 72 ± 18. This response was less consistent than rapid excitation and no dose-dependency was observed. Unit responses to a standard dose of 10 μg are shown in figure 4.4. The majority of units displayed both types of response, with only one unit showing only the delayed effect in response to a single 10 μg dose.

Arthritic joints

From nine experiments eleven slowly adapting mechanonoreceptors with a mean afferent fibre conduction velocity of 2.1 ± 1.0 ms^{-1} (range: 0.3 - 7.8 ms^{-1}) were examined. These units had a mean resting discharge of 0.8 ± 0.2 i.p.s..

Capsaicin (1 - 10 μg, i.a.) consistently activated all of the units tested (table 4.1). The responses were of short latency (0.7 ± 0.2 s), short duration (22 ± 4 s) and high discharge frequency. The mean peak discharge, obtained over a 15 s time period, was 5.4 ± 1.3 i.p.s., and

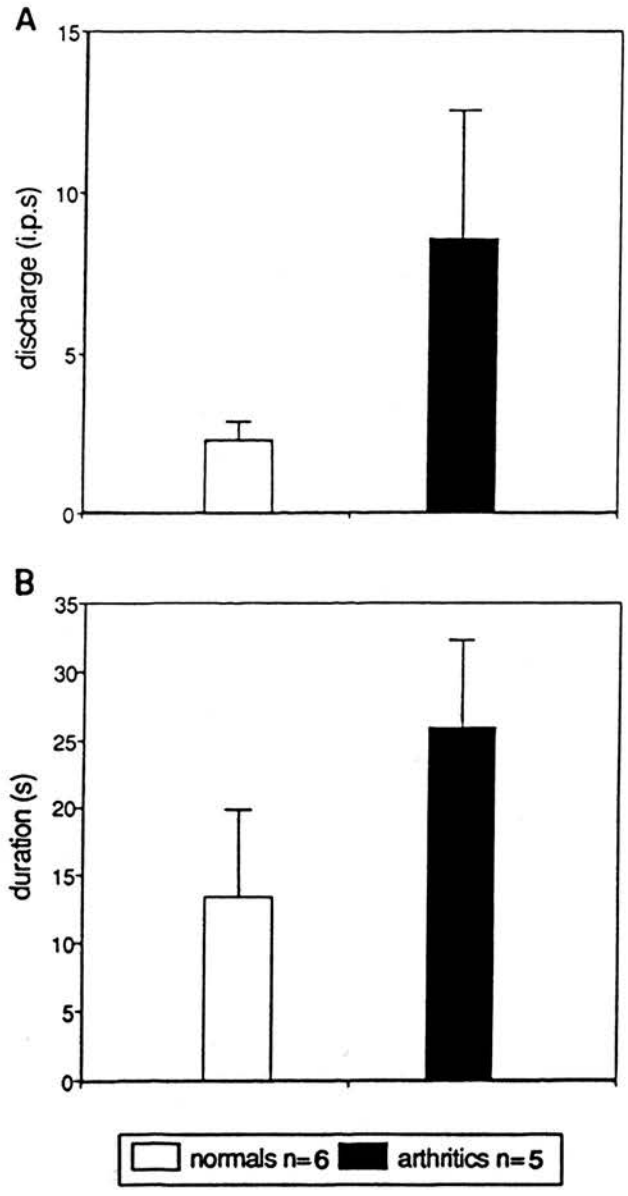


Fig. 4.2 Mean peak response and duration of rapid excitation evoked by a standard dose of 10 μg capsaicin (i.a.) in normal and arthritic joints. Not all units were tested with this dose of capsaicin. (A) The peak discharge, obtained over a 15 s recording period, was greater, although not significantly ($p < 0.05$, Wilcoxon), in arthritic rats compared with normals. (B) The duration of the rapid excitation was also marginally longer for arthritics.

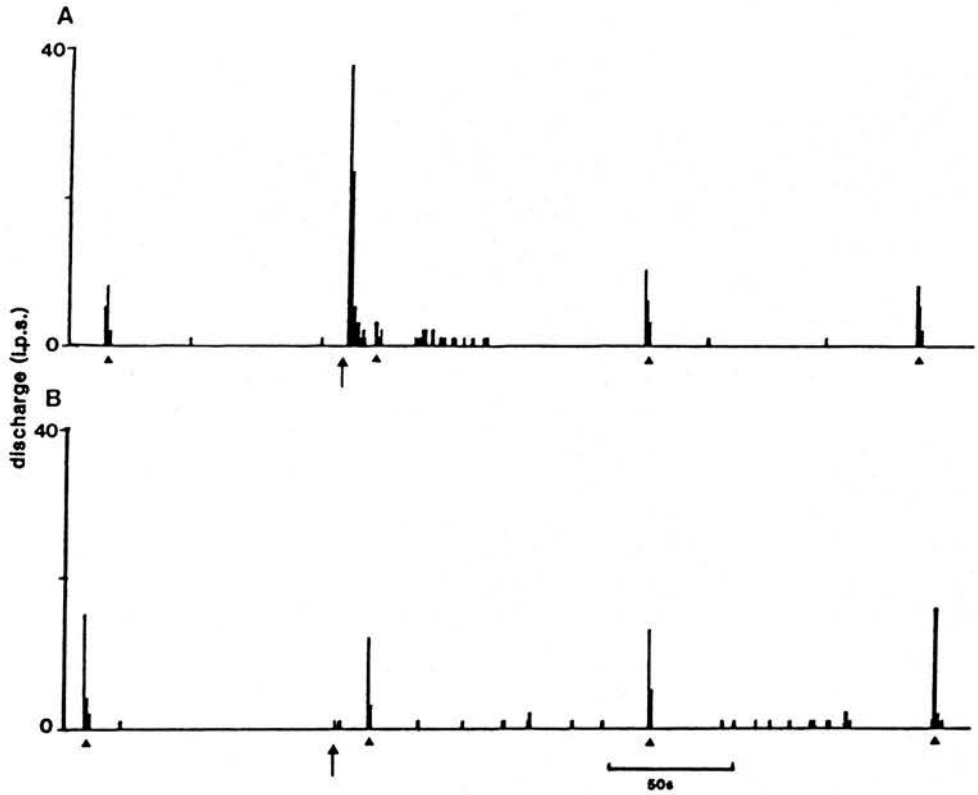


Fig 4.3 Computer-generated plot illustrating the response of a slowly adapting mechanoreceptor unit from a normal joint to repeated i.a. injection of 5 μg capsaicin (given at arrow). Each collection bin (bar) represents a 1 s time period. Responses to mechanical stimuli (applied at arrow heads) were not increased by capsaicin. This unit had an afferent fibre conduction velocity of 0.8 ms^{-1}. Consecutively labelled graphs (A) and (B) represent consecutive injections of capsaicin.

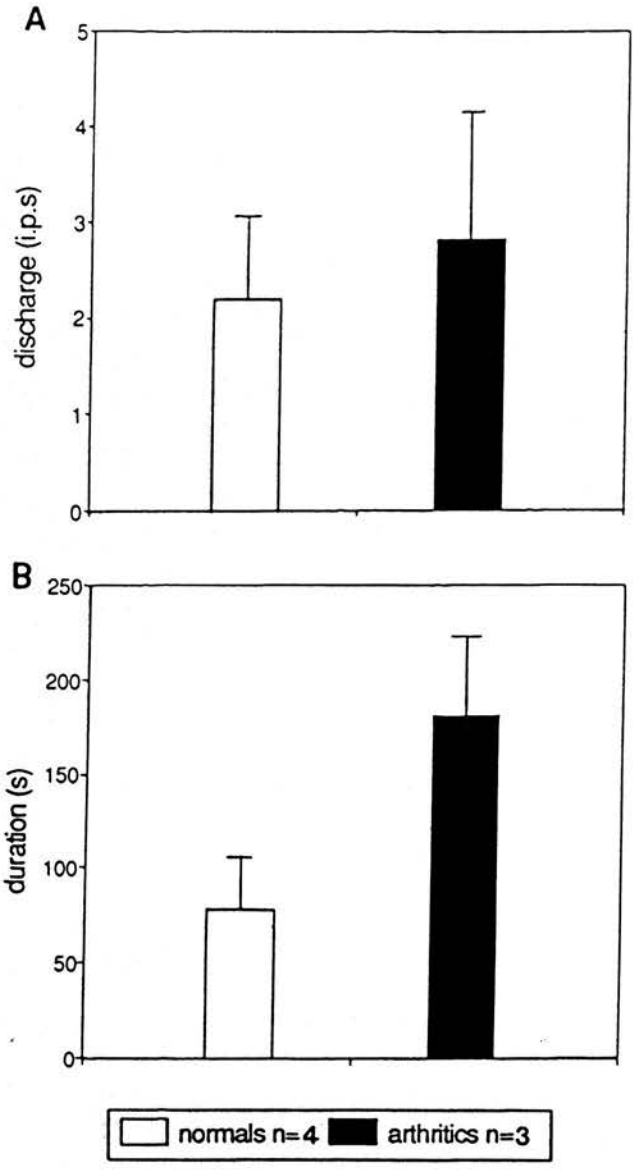


Fig. 4.4 Mean peak response and duration of delayed excitation evoked by a standard dose of 10 μg capsaicin (i.a.) in normal and arthritic joints. Not all units were tested with this dose of capsaicin. (A) The peak discharge, obtained over a 15 s recording period, was marginally greater in arthritic rats compared with normals. (B) The duration of the rapid excitation was also longer, although not significantly ($p < 0.05$, Wilcoxon) for arthritics.

the mean total number of evoked action potentials above basal discharge (delta Σx) was 106 ± 33. This effect is greater than that seen in receptors from normal joints for the same dose levels of capsaicin. Unit responses to a standard dose of 10 μg are shown in figure 4.2. Repeated injections of 1 - 10 μg doses at intervals of 15 minutes evoked a consistent response. Application at shorter intervals caused a reduction of the response to develop. Dose-dependent effects were observed in three units.

In addition to rapid excitation, capsaicin also evoked a delayed (51 ± 8.6 s), longer lasting (195 ± 67 s) increase in activity in seven (64%) units. The mean peak discharge, obtained over a 15 s time interval, was 2.4 ± 0.6 i.p.s., and the mean total number of action potentials evoked above basal discharge (delta Σx) was 195 ± 67. This effect is of greater amplitude and duration to that seen in normal joints. Unit responses to a standard dose of 10 μg are shown in figure 4.4. The delayed response was less consistent than rapid excition and it was not possible to determine dose-dependency. The rapid excitation was seen for ten (91%) of eleven units and the delayed response for seven (64%) of eleven units. One unit gave only the delayed response and four units only the rapid excitation.

4.2.1.2 Effects on mechanoreceptor responsiveness

Normal joints

From five experiments seven units were examined for the affects of capsaicin (1 - 10 μg, i.a.) on their responsiveness to a standard

mechanical stimulus applied once every 2 minutes, responsiveness was increased (sensitization) in four (57%) (see fig 4.1). Mechanical sensitization occurred after a delay of 15 seconds in three units and 2 minutes in the fourth, and lasted for one to two stimuli. A summary of unit responses is shown in figure 4.5. The mean peak response was 162% of the pre-injection control. Dose-dependency of the effect was seen in three units.

Arthritic joints

In nine units from six experiments, capsaicin (1 - 10 μg, i.a.) caused a sensitization to mechanical stimuli in seven (77%) (fig 4.4). A delay of 15 seconds in five units and 2 minutes in two units occurred before the onset of the response which lasted for one to four stimuli (see fig. 4.1). The mean peak response was 161% of the pre-injection control. Dose-dependency was not seen in these units. In three of the mechanoreceptors sensitized by 1 - 5 μg capsaicin, a subsequent injection of 10 μg caused a short lived reduction in responsiveness to mechanical stimuli. A further unit gave only this reduction in responsiveness following capsaicin injection.

4.2.1.3 Excitation of chemosensitive units

Normal joints

In this study four recordings consisting of between one and three different action potential spikes were obtained from units, often with a

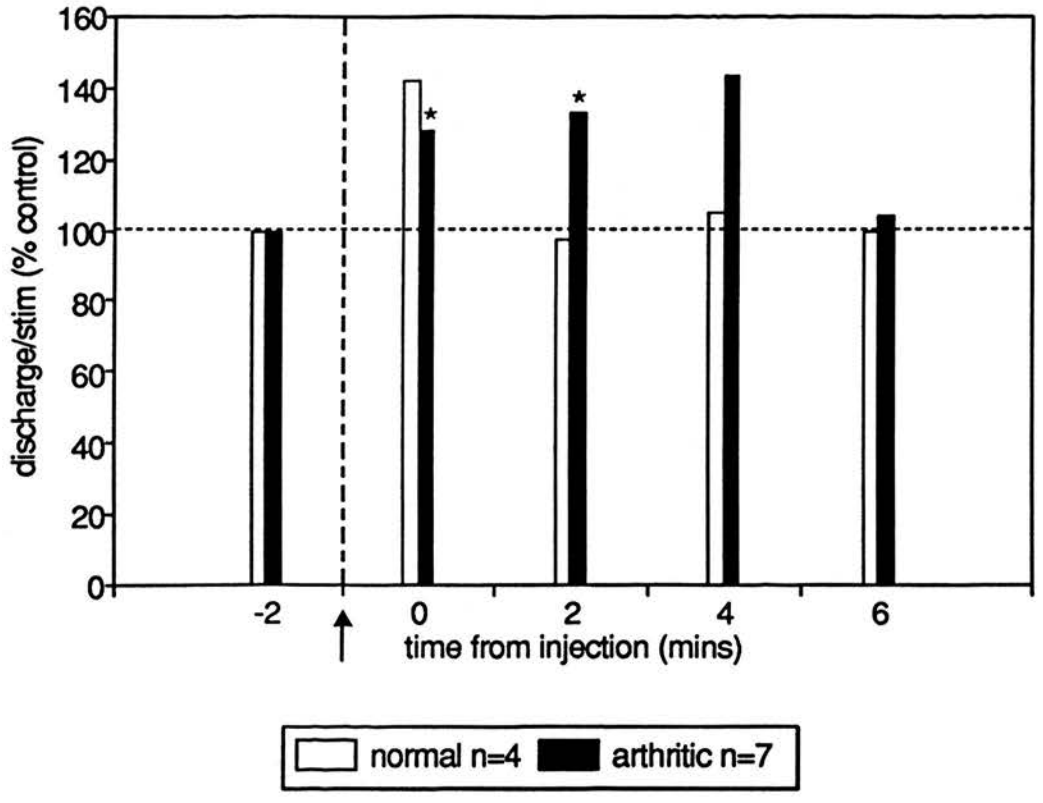


Fig. 4.5 Pooled data showing changes in mechanoreceptor responsiveness caused by the i.a. injection of capsaicin in normal and arthritic joints. Changes in responsiveness of the four mechanoreceptors from normal joints and seven mechanoreceptors from arthritic joints that were briefly sensitized to mechanical stimuli following injection of capsaicin (at arrow). For these units responses are shown for a median dose of 5 μg capsaicin for normal and arthritic groups. Each bar represents the mean response of five units to mechanical stimuli applied every 2 mins. Sensitization in arthritic joints was of longer duration than that seen in normals. Significant differences from control pre-injection responses are shown as * when $p < 0.05$ (Wilcoxon).

low level of background activity (mean; 1.2 ± 0.6 i.p.s), for which no mechanoreceptive fields could be found. Their action potential spike shape characteristics were similar to those of identified C-fibre afferents, in terms of spike width and amplitude (see Section III).

Capsaicin (1 - 10 μg, i.a.) consistently activated all of the units tested (table 4.1). In all recordings injection of capsaicin evoked responses of short latency (0.8 ± 0.4 s), short duration (22 ± 9.6 s) and high discharge frequency. The mean peak discharge, obtained over a 15 second time period, was 4.7 ± 2.0 i.p.s.. Capsaicin also evoked a delayed (33 ± 14 s), longer lasting (79 ± 10 s) increase in activity in three (75%) recordings. The mean peak discharge of the delayed excitation, obtained over a 15 s time interval, was 3.2 ± 2.2 i.p.s..

Arthritic joints

Six recordings, consisting of between one and three different action potential spikes, were obtained from units for which no mechanoreceptive fields could be found. Their action potential spike shape characteristics were similar to those of identified C fibre afferents (see Section III), and their mean rate of resting discharge before the injection of capsicin was 1.3 ± 0.3 i.p.s..

In all recordings injection of capsaicin (1 - 10 μg, i.a.) evoked responses of short latency (1.1 ± 0.2 s), short duration (18 ± 3.3 s) and high discharge frequency (table 4.1). The mean peak discharge, obtained over a 15 s time period, was 8.3 ± 1.9 i.p.s..

The delayed (44 ± 10 s), longer lasting (149 ± 33 s) increase in activity was obtained in four (67%) recordings. The mean peak discharge,

obtained over a 15 s time interval, was 4 ± 0.6 i.p.s..

4.2.1.4 Effects of other algogens on mechanoreceptors excited by capsaicin

The excitatory effects of capsaicin were examined in experiments in which other putative mediator substances were also being tested. A summary of the results obtained is shown in table 4.2. From this information it can be seen that capsaicin excited all of the units excited by bradykinin (0.1 - 40 μg, i.a.), 5-HT (1 - 100 μg, i.a.), PGE_2 (0.03 - 3 μg, i.a.) and the selective PGI_2 (IP-) receptor agonist cicaprost (0.01 - 5 μg, i.a.). Two units not excited by bradykinin and three units not excited by PGE_2 were sensitive to capsaicin.

4.2.2 In vitro electrophysiology

4.2.2.1 Excitatory effects on mechanoreceptors

From six experiments on normal joints, six high-threshold slowly adapting mechanoreceptors with action potential spike shape characteristics similar to those of identified C fibres were examined. These units had a mean ongoing resting discharge of 0.07 ± 0.05 i.p.s..

Capsaicin (0.01 - 10 μg, i.a.) activated five (83%) of the six units tested (table 4.3). Injection of capsaicin evoked responses of short latency (1.8 ± 0.3 s), short duration (25 ± 9.4 s) and high discharge frequency (see fig. 4.6). The mean peak discharge, obtained over a 15 s time period, was 2.4 ± 1.0 i.p.s., and the mean total number of evoked

Table 4.2 Summary of the number of A-delta and C fibre mechanonociceptors excited by i.a. injection of capsaicin, bradykinin, 5-HT, PGE_2 or cicaprost.

	number of responsive units			
	NORMAL		ARTHRITIC	
C.V.	C 0.5-2.5 ms-1	A-delta 5.3-16.6 ms-1	C 0.3-1.25 ms-1	A-delta 5.3-7.8 ms-1
capsaicin (1 - 10 μg)	10/10 (100%)	3/3 (100%)	9/9 (100%)	2/2 (100%)
bradykinin (0.1 - 40 μg)	2/2 (100%)	2/3 (67%)	3/4 (75%)	-
5-HT (1 - 100 μg)	7/7 (100%)	2/2 (100%)	4/4 (100%)	2/2 (100%)
PGE_2 (0.03 - 3 μg)	0/1 (0%)	-	1/2 (50%)	-
cicaprost (0.01 - 5 μg)	2/2 (100%)	1/1 (100%)	4/4 (100%)	-

C.V. = conduction velocity range

Table 4.3 Summary of the number of mechanonociceptors and chemosensitive units responding to capsaicin *in vitro*.

UNITS	n	rapid excitation	delayed excitation
mechanoreceptors	6	5 (83%)	3 (50%)
chemosensitive	9	9 (100%)	3 (33%)

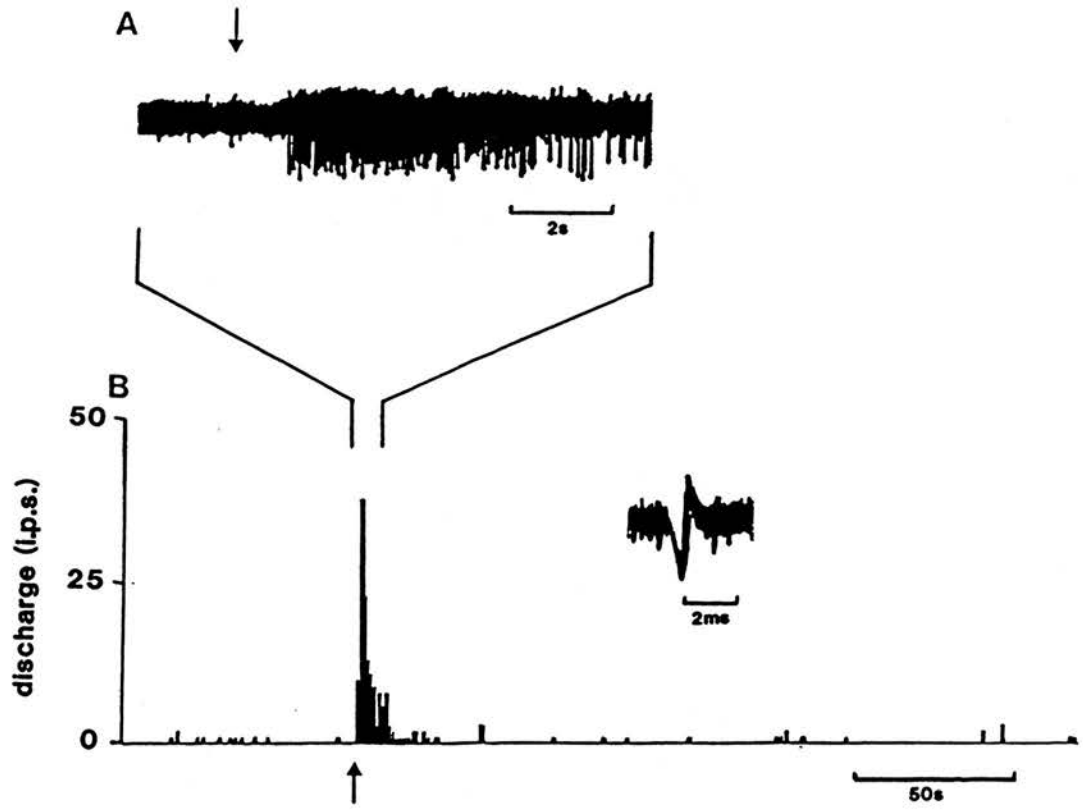


Fig. 4.6 Response of an articular mechanonociceptor to i.a. injection of capsaicin <u>in vitro</u>. (A) Neurogram of the discharge pattern of rapid excitation evoked by injection of capsaicin (0.1 μg, i.a.). (B) Computer-generated plot of the discharge evoked by the same injection of capsaicin shown in the neurogram. The time period corresponding to that in the neurogram is indicated above the graph. Each bin (bar) represents a 1 s time period. The inset shows thirty superimposed triggered oscilloscope sweeps of the recorded unit action potential.

action potentials above basal discharge (delta Σx) was 23 ± 9.

In addition to rapid excitation, capsaicin also evoked a delayed (68 ± 12 s), longer lasting (206 ± 61 s) increase in activity in three (50%) units (Fig. 4.5). The mean peak discharge, obtained over a 15 s time interval, was 0.5 ± 0.1 i.p.s., and the mean total number of action potentials evoked above basal discharge (delta Σx) was 12 ± 2. Three units displayed both types of response, one unit only the rapid excitation and one unit only the delayed response (Fig.4.7).

4.2.2.2 Excitation of chemosensitive units

In this series of experiments nine recordings consisting of between one and three different action potential spikes were obtained from units, for which no mechanoreceptive fields could be found. Their action potential spike shape characteristics were similar to those of identified C-fibre afferents, and they had a resting discharge of 0.6 ± 0.1 i.p.s..

Capsaicin (1 - 10 μg, i.a.) consistently activated all of the units tested (table 4.3). In all recordings injection of capsaicin evoked responses of short latency (1.9 ± 0.3 s), short duration (12 ± 2 s) and high discharge frequency. The mean peak discharge, obtained over a 15 s time period, was 4.8 ± 1.4 i.p.s.. Capsaicin also evoked a delayed (42 ± 10 s), longer lasting (118 ± 22 s) increase in activity in three (75%) recordings. The mean peak discharge, obtained over a 15 s time interval, was 1.3 ± 0.2 i.p.s..

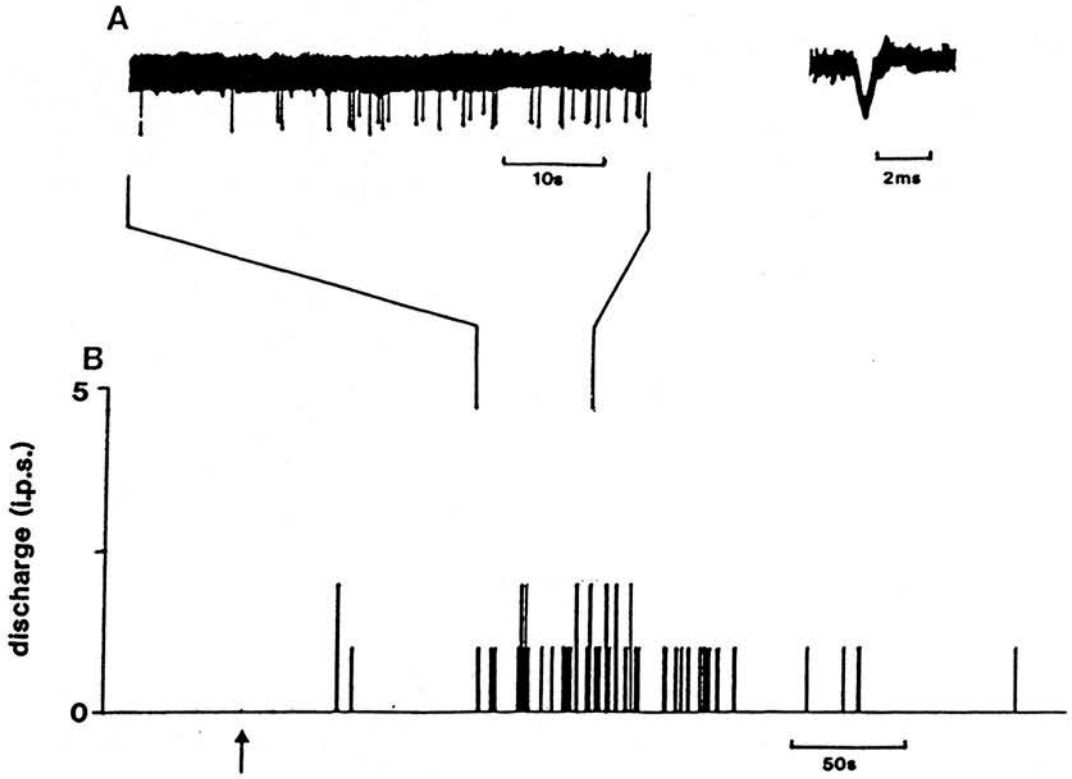


Fig. 4.7 Response of an articular mechanonociceptor to i.a. injection of capsaicin <u>in vitro</u>. (A) Neurogram of the discharge pattern of delayed excitation evoked by injection of capsaicin (1 μg, i.a.). The inset trace on the right shows thirty superimposed triggered oscilloscope sweeps of the recorded unit action potential. (B) Computer-generated plot of the discharge evoked by the same injection of capsaicin shown in the neurogram. The time period corresponding to that in the neurogram is indicated above the graph. Each bin (bar) represents a 1 s time period.

4.2.2.3 Effects of other algogens on mechanoreceptors excited by capsaicin

The excitatory effects of capsaicin were examined in experiments in which other putative mediator substances were also being tested. A summary of the results is shown in table 4.4. Capsaicin excited all but one of the units excited by bradykinin (0.1 - 10 μg, i.a.), 5-HT (0.01 - 100 μg, i.a.), PGE_2 (0.01 - 10 μg, i.a.) or the selective PGI_2 (IP-) receptor agonist cicaprost (0.01 - 1 μg, i.a.). Three units not excited by bradykinin, two units not excited by PGE_2, and one unit not excited by 5-HT were sensitive to capsaicin.

Table 4.4 Summary of the number of mechanonociceptors excited by i.a. injection of capsaicin, bradykinin, 5-HT, PGE2 or cicaprost in vitro.

treatment	dose	number of responsive units
capsaicin	0.01 - 10 μg	5/6 (83%)
bradykinin	0.1 - 10 μg	1/5 (20%)
5-HT	0.01 - 100 μg	4/5 (80%)
PGE_2	0.01 - 10 μg	0/3 (0%)
cicaprost	0.01 - 1 μg	3/3 (100%)

4.3 DISCUSSION

4.3.1 Normal joints

These results show that capsaicin potently excites articular high threshold mechanoreceptors with afferent fibre conduction velocities in the C fibre and fine A-delta range. This differs from the situation reported for skin where only A-delta and C-polymodal nociceptors were excited, with C and A-delta high threshold mechanoreceptors being insensitive to the drug (Foster & Ramage, 1981; Kenins, 1982; Szolcsanyi, 1987; Szolcsanyi et al., 1988). The afferent axons of all the receptors studied here, have previously been reported to be unmyelinated at their peripheral regions, and as such might be expected to be equally sensitive to chemical stimuli (Paintal, 1971). Units with conduction velocities in the C fibre or A-delta range were equally responsive to capsaicin, suggesting that they do not differ functionally in this respect. Both types of unit also have similar mechanical response characteristics (see Section III and Guilbaud et al., 1985). The sensory receptors examined here are normally insensitive to noxious thermal stimuli (Guilbaud et al., 1985), and therefore, cannot be classified as polymodal. Thus, it can be concluded that the receptors described in the present series of studies constitute a population of C and fine A-delta articular nociceptors responding selectively, under non-pathological conditions, to chemical and strong mechanical stimuli.

Capsaicin-evoked responses of rapid onset and with high discharge rates have been reported by other workers (Foster & Ramage, 1981; Kenins, 1982; Szolcsanyi, 1987), whereas the longer latency response

observed in the present study have not previously been described. This response was seen both *in vivo* and *in vitro*, eliminating the possibility of the involvement of second pass effects, systemic release of blood borne mediators, or reflex effects mediated through the spinal cord. The minimally effective dose of capsaicin *in vitro* was at least ten times lower than that required *in vivo*. As the mechanonociceptors under study did not have any greater mechanical sensitivity *in vitro* (see Section III), this difference may be due to an improved access of capsaicin to these receptors via the vasculature. Although no obvoius pathology was seen in these experiments, damage to the vessel walls or endothelium caused by the perfusion could be responsible for increasing their permeability.

Capsaicin-induced sensitization of nociceptors, which follows a similar time course to the delayed excitation, has not previously been reported in electrophysiological studies. Capsaicin is known to induce the release of neuropeptides such as substance P from sensory terminals in the periphery (Hoover, 1987; Renzi et al., 1988; Manzini et al., 1989), which may in turn release other mediators such as the prostanoids or 5-HT (see Holzer, 1988). Thus, it is possible that delayed excitation or mechanical sensitization occur secondary to the production of other local effector substances. Alternatively, increased levels of intracellular Ca^{2+}, resulting from the opening of cation channels in the cell membrane (Bevan & Forbes, 1988), may result in the activation of second messenger systems involved in the control of neuronal excitability. This mechanism could also account for the delayed and sustained nature of these responses. Further investigations using selective neuropeptide antagonists, inhibitors of arachidonic acid

metabolism and manipulations of second messenger mechanisms will be necessary in order to determine the significance of these systems in the delayed effects of capsaicin.

Desensitization of nociceptor responses to capsaicin seen in the present study have also been reported extensively by other workers (Szolcsanyi, 1987; Belmonte et al., 1988; Bettaney et al., 1988a). In the rabbit skin (Szolcsanyi, 1987) and cat cornea (Belmonte et al., 1988), high doses of capsaicin non-selectively abolished the responses of C-polymodal nociceptors to mechanical, chemical and thermal stimuli. However, in the neonatal rat spinal cord-tail preparation in vitro, using lower doses, selective desensitization to capsaicin was achieved, with responses to mechanical and thermal stimuli, and bradykinin, being unaffected. This finding is supported by results from the present series of experiments where single injections of low doses had no affect on nociceptor reponses to mechanical or further chemical stimuli.

Capsaicin-induced excitation of afferent units for which no mechanosensitive receptive fields could be found had the same response pattern as seen for identified mechanoreceptors. These sensory receptors are also excited by bradykinin, 5-HT and the prostanoids (see Sections V-VII). Other workers have recently identified mechanically and thermally insensitive cutaneous C fibre units which are excited by chemical stimuli (Meyer & Campbell, 1988; Davies et al., 1989). These specifically 'chemosensitive' sensory receptors may have an important role to play during inflammation (see Section III).

4.3.2 Arthritic joints

Both rapid and delayed excitation of receptors was observed in recordings from arthritic joints. The rapid response was of greater magnitude and longer duration, suggesting that these receptors had a higher level of excitability than those in normal joints. Delayed excitation and capsaicin-induced sensitization to mechanical stimuli were also of longer duration. Enhanced responsiveness to capsaicin may result from the actions of various inflammatory mediators on sensory receptors in the chronically inflamed joint. In the neonatal rat spinal cord-tail preparation *in vitro* PGE_1 and PGE_2 have been shown to markedly potentiate capsaicin-induced ventral root depolarization via an effect on peripheral nerve endings (Yanagisawa et al., 1986). PGE_2 has also been shown to potentiate the excitatory effects of capsaicin in skin, as recorded from thalamic neurones in the cat (Andoh et al., 1982). This finding suggests that prostanoids may be at least partly responsible for the enhancement of the effects of capsaicin seen in arthritic joints (see also Sections VII). If the delayed effects of capsaicin observed in the present study are produced secondary to the release of other substances, then their enhancement in chronically inflamed tissues may occur as a result of increased release of these substances. As discussed in Section III of this thesis levels of substance P in sensory neurones are elevated as a result of the arthritic lesion, and therefore, more will be available for release when the nerve is depolarized by capsaicin. Furthermore, the greater availability of a variety of cell types from which other mediators could be released, may add to this effect.

Depression of mechanoreceptor responsiveness seen in a small number of units from arthritic rats may occur as a result of the nonselective desensitizing effect of capsaicin reported by other workers (Szolcsanyi, 1987; Belmonte et al., 1988; Bettaney et al., 1988a). Mechanoreceptor desensitization was only observed in units from arthritic joints, and may be related to the enhanced effect of capsaicin in these tissues. Desensitization of C-polymodal nociceptors has been shown not to be a general phenomena. Thus, in the rabbit skin desensitization of a single nociceptor to all forms of stimuli was not observed (Szolcsanyi, 1987). This finding suggests that desensitization does not occur as a result of action potential blockade, a known property of high dose capsaicin (Petsche et al., 1983; Handwerker et al., 1984; Lynn et al., 1984), but rather results from effects on the impulse-generating mechanisms for the different modalities (Szolcsanyi, 1987). Anti-nociceptive effects of capsaicin, mediated via central and peripheral terminals, have recently been demonstrated (Dickenson et al., 1990). It has been suggested that these effects may be mediated via long-lasting inhibition of voltage-gated Ca^{2+} channels induced by capsaicin (Bevan & Szolcsanyi, 1990).

4.4 CONCLUSIONS

Results from the present study suggest that capsaicin can be used as a selective pharmacological probe to test the accessibility of chemosensitive nociceptors via the blood supply. Its usefulness in neuropharmacological experiments is supported by the finding that single, low dose injections do not have any long lasting effect on

mechanonociceptor responsiveness. The longer duration effects of capsaicin on mechanonociceptors from arthritic joints, suggests that these receptors are more sensitive to chemical stimuli than those from normal joints. This is supported by findings reported in Section VI showing that 5-HT also has greater effects on mechanonociceptors from arthritic joints.

Electrophysiological studies in vitro have shown that tarsal joint mechanonociceptors function normally under conditions of combined superfusion and slow perfusion, responses to mechanical (see Section III) and chemical stimuli being maintained for periods of several hours.

SECTION V

EFFECTS OF BRADYKININ ON ARTICULAR SENSORY RECEPTORS IN THE RAT ANKLE JOINT

SECTION V

EFFECTS OF BRADYKININ ON ARTICULAR SENSORY RECEPTORS IN THE RAT ANKLE JOINT

5.1 INTRODUCTION

The generation of plasma kinins is increased markedly during inflammation (Lewis, 1970). Local production of bradykinin in inflammatory exudates causes vasodilation and tissue oedema (Regoli & Barabe, 1980), and bradykinin is also a powerful algesic agent causing intense, burning pain when applied to the exposed blister base at concentrations below 0.1 μM (Keele & Armstrong, 1964). Bradykinin is also known to cause pain in man when injected intra-arterially (Burch & Pasquale, 1962; Coffman, 1966;), subdermally (Lim et al., 1967; Ferreira, 1972) or intra-abdominally (Lim et al., 1967). Furthermore, pseudoaffective responses in animals are produced by injection of bradykinin intra-arterially (Guzman et al., 1962; Hashimoto et al. 1964; Ferreira et al., 1973) or intra-articularly (Melmon et al., 1967; Moncada et al., 1975).

Electrophysiological studies have demonstrated that bradykinin excites nociceptors from a variety of tissues including the skin (Beck & Handwerker, 1974; Chahl & Iggo, 1977; Fjallbrant & Iggo, 1961), viscera (Haupt et al., 1983; Kumazawa & Mizumura, 1976), muscle (Mense & Schmidt, 1974; Kumazawa & Mizumura, 1977b) and joints (Kanaka et al. 1985). Bradykinin-induced sensitization of muscular nociceptors to

mechanical stimuli has also been shown (Mense & Meyer, 1987).

The present study examined the effects of bradykinin on articular nociceptive sensory receptors in the ankle joint of the rat. Assessment of bradykinin's actions on sensory receptors in arthritic joints was also carried out to determine the actions of the peptide on mechanonociceptors during chronic inflammation.

5.2 RESULTS

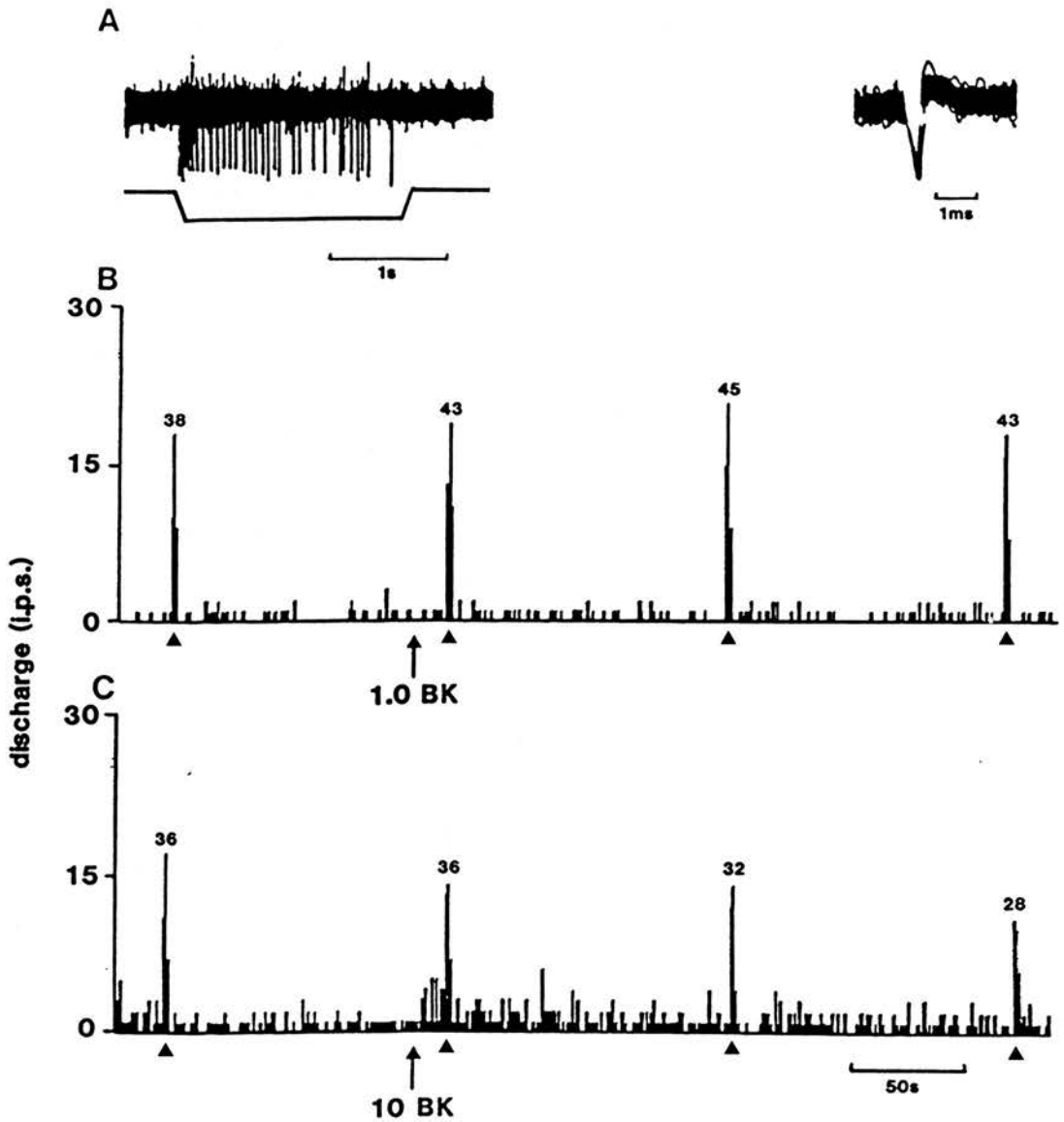
5.2.1 Electrophysiology in vivo

The effects of intra-arterial injections of bradykinin on articular mechanonociceptors were examined in normal and arthritic joints. Bradykinin excited and sensitized mechanosensitive units from the ankle joints of both groups of animals (fig. 5.1). No difference in responsiveness between receptors with C or A-delta afferent fibres was found. Excitation of units for which no mechanoreceptive fields could be found was also produced. However, as their response characteristics were similar to those of the mechanoreceptors which could be identified more fully, responses of these units were not analysed routinely in this series of experiments.

Normal joints

In twenty four experiments using normal rats, thirty three high-threshold slowly adapting mechanoreceptors with A-delta and C fibre

Fig. 5.1 Computer-generated plots showing bradykinin-induced activation and increased responsiveness to mechanical stimuli given once every two minutes. (A) Neurogram showing mechanically evoked activity in the high threshold mechanoreceptor unit with an afferent fibre conduction velocity of 0.4 ms-1. Dynamic and slowly adapting responses can be seen. The inset shows thirty superimposed triggered oscilloscope sweeps of the action potential generated by stimulation of the mechanoreceptor. (B) Mechanical sensitization caused by injection of 1 μg bradykinin (at arrow). The number of action potentials evoked by each mechanical stimulus (applied at arrowheads) is given above each response. (C) Mechanoreceptor excitation evoked by injection of 10 μg bradykinin (at arrow). Mechanoreceptor sensitization was not seen with this higher dose due to desensitization of the reponse.


A
1s
1ms
B
30
15
0
38
43
45
43
1.0 BK
C
30
15
0
36
36
32
28
50s
10 BK
discharge (i.p.s.)

afferent (mean afferent fibre conduction velocity: 2.0 ± 0.5 ms^{-1}, range: 0.38 - 8.5 ms^{-1}) were examined for their responsiveness to bradykinin. Eleven (33%) of these units had an ongoing spontaneous discharge of 0.4 ± 0.1 i.p.s. under resting conditions.

Arthritic joints

In ten experiments thirteen slowly adapting mechanoreceptors with C and A-delta afferent fibres (1.2 ± 0.6 ms^{-1}, range: 0.3 - 4.0 ms^{-1}) were examined for their responsiveness to bradykinin using arthritic joints. In contrast to the small number of acive units seen in normal joints eleven (85%) of these units had a resting discharge of 1.4 ± 0.4 i.p.s..

5.2.1.1 Bradykinin-induced excitation

Normal joints

Injection of bradykinin (0.1 - 40 μg, i.a.) excited twenty five (76%) of the thirty three units. The minimal effective dose ranged from 0.1 - 10 μg for the twenty one units examined to determine this parameter (fig. 5.2). In four units tested with only a single dose of 40 μg, three (75%) responded with a prolonged, large increase in discharge. Excitation had a mean latency to onset of 65 ± 11 seconds and a mean duration of 171 ± 18 seconds.

Examination of dose-dependency (0.1 - 10 μg, i.a.) was carried out on nineteen units of which seven (37%) displayed a clear dose-response relationship and twelve (63%) did not (fig. 5.3). Measurements of peak

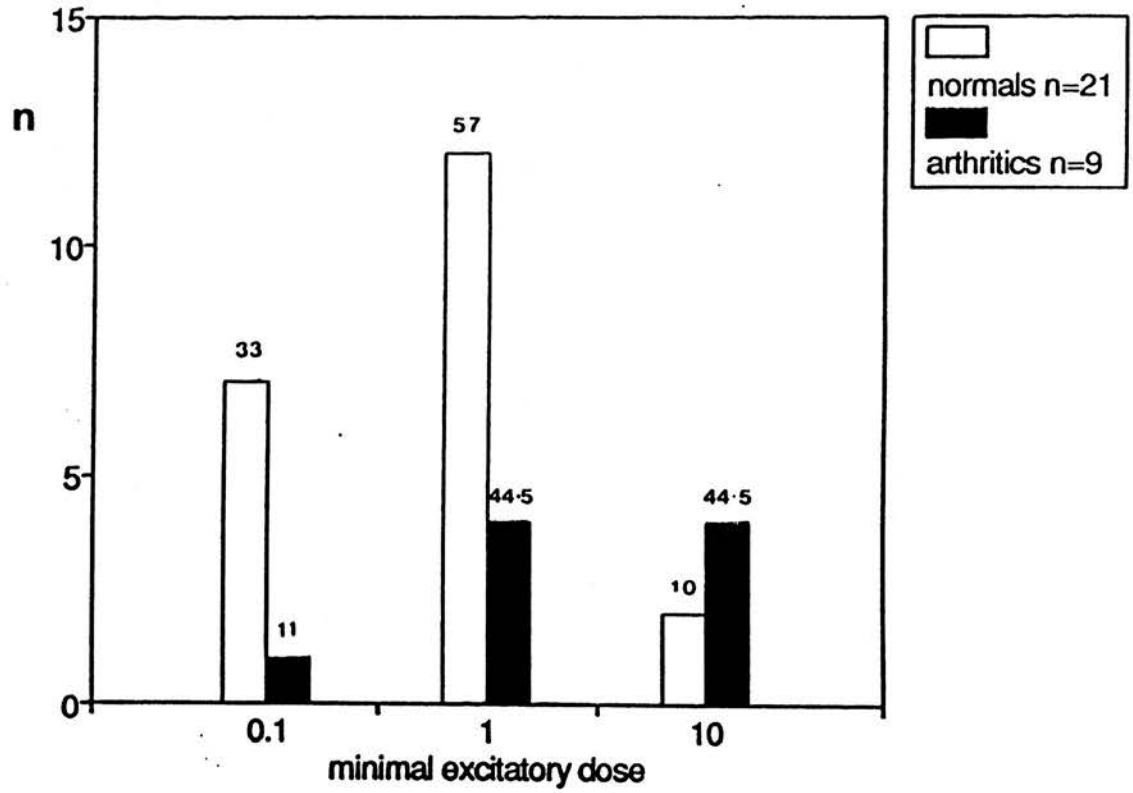


Fig. 5.2 Minimal effective doses of bradykinin causing excitation of high threshold mechanoreceptors from normal (n=21) and arthritic (n=9) joints. The number of units (n) with minimal effective doses of 0.1, 1.0 and 10 μg are shown. The percentage of excitable units giving an initial response to each dose is given above the appropriate bar. As can be seen from the graph, a greater proportion of units from normal joints were excited by a lower minimal effective dose than were units from arthritic joints.

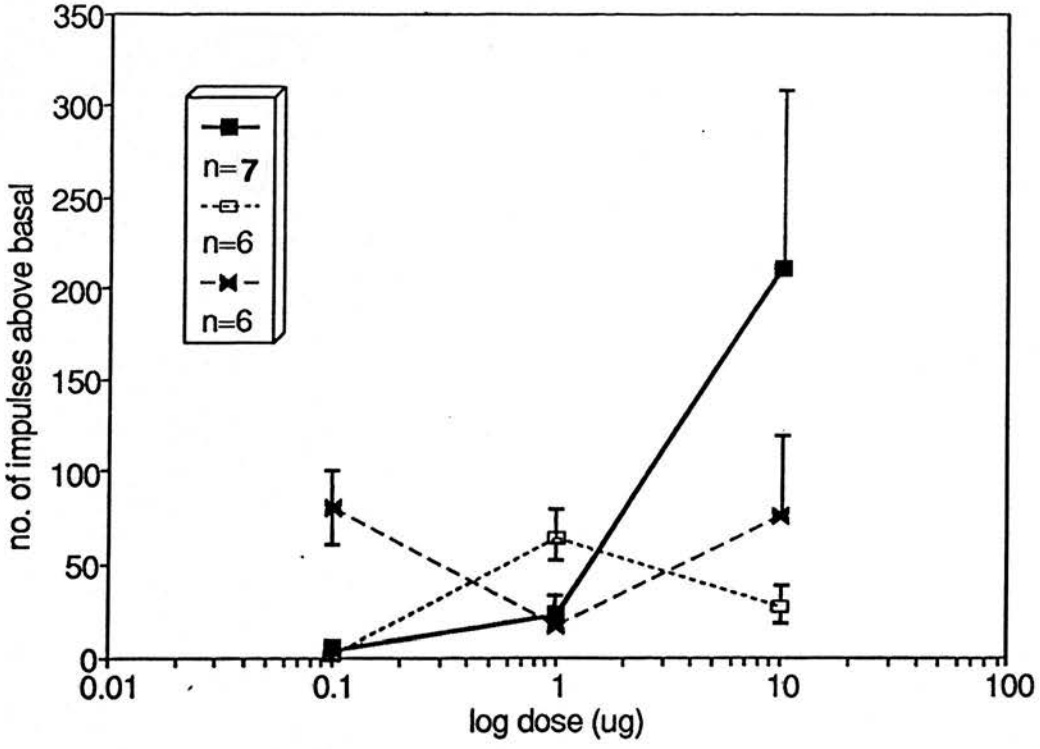


Fig. 5.3 Dose-dependent excitatory effects of bradykinin on high thresholds **mechanoreceptors from normal joints. Clear dose dependent effects were seen in seven units (■). These units had a mean afferent fibre conduction velocity of 1.3 ± 0.4 ms-1. Desensitization occurred at the highest dose of 10 μg in six units (□), and further six units no dose dependent effect was evident (✕). These twelve units have a mean afferent fibre conduction velocity of 2.5 ± 0.7 ms-1.**

discharge alone did not consistently reveal a dose-dependent effect of bradykinin, as duration of response also increased with the dose administered. Thus, responses are expressed as the total number of impulses above basal discharge produced for the duration of the effect (integrated response given as delta Σx as detailed in Section II). The dose-response curve for the twelve units not responding in a dose-dependent manner was flat in appearance with a low maximum (fig. 5.3). The mean maximal integrated response for units examined for dose-dependency (0.1 - 10 μg) was 157 ± 48 impulses above basal (delta Σx), with a mean peak discharge obtained over a fifteen second period of 2.9 ± 1.1 i.p.s.. Maximal responses were produced by a median dose of 1.0 μg (see table 5.1).

The four units tested with only high dose (40 μg) bradykinin gave a mean maximal response of 238 ± 83 impulses (delta Σx), with a mean peak discharge of 2.9 ± 0.4 i.p.s. over a 15 s time period. In two units for which a second injection of 40 μg bradykinin was given, tachyphylaxis of the response was evident.

Arthritic joints

Injection of bradykinin (0.1 - 40 μg, i.a.) excited ten (77%) of the thirteen units. The minimal dose for receptor excitation ranged from 0.1 - 10 μg (fig. 5.2). Excitation had a mean latency to onset of 27 ± 8 seconds and a mean duration of 120 ± 20 seconds.

Maximal responses were evoked by a median dose of 1.0 μg for units tested with doses of 0.1 - 10 μg. A mean maximal integrated response of 209 ± 85 impulses above basal discharge (Σx) was obtained, the mean peak

Table 5.1 Maximal bradykinin-induced excitatory response in normal and arthritic rats.

	NORMALS n=19	ARTHRITICS n=10
mean unit max (delta Σx)	157 ± 48	209 ± 85 **
latency (s)	71 ± 24 **	27 ± 8
duration (s)	256 ± 40 **	120 ± 20

** significantly greater value ($p < 0.01$)

discharge over a 15 s time period being 4.5 ± 1.2 i.p.s. (table 5.1). Responses to bradykinin were dose-dependent in five of the ten units, the remaining ten responsive units displaying either an 'all-or-none' type response or reduced responsiveness with increasing dose.

5.2.1.2 Effects on mechanonociceptor responsiveness

Normal joints

Twenty six mechanonociceptors were examined for the effects of bradykinin (0.1 - 40 μg, i.a.) on their responsiveness to controlled mechanical stimuli. Increased responsiveness to mechanical stimuli (sensitization) following injection of bradykinin was seen for eighteen (70%) of these units. There was no correlation between units that were excited by bradykinin and those that were sensitized to mechanical stimuli. Thus, four units (15%) which were not excited by bradykinin were also unaffected in their responsiveness to mechanical stimuli, another four (15%) units sensitized to mechanical stimuli were not excited by the drug, and a further four units excited by bradykinin were not sensitized to mechanical stimuli.

The minimal dose causing receptor sensitization was between 0.1 and 10 μg (fig. 5.4). Bradykinin-induced changes in receptor responsiveness had a latency to onset of between 15 seconds and 6 minutes (mean: 2.5 ± 0.5 minutes), and lasted for between one and ten mechanical stimuli (mean duration: 6.5 ± 1.0 minutes).

Thirteen responsive units were examined for the construction of a dose response curve to bradykinin (0.1 - 10 μg, i.a.), and of these only four (31%) responded in a dose-dependent manner (Fig. 5.5). In the remaining

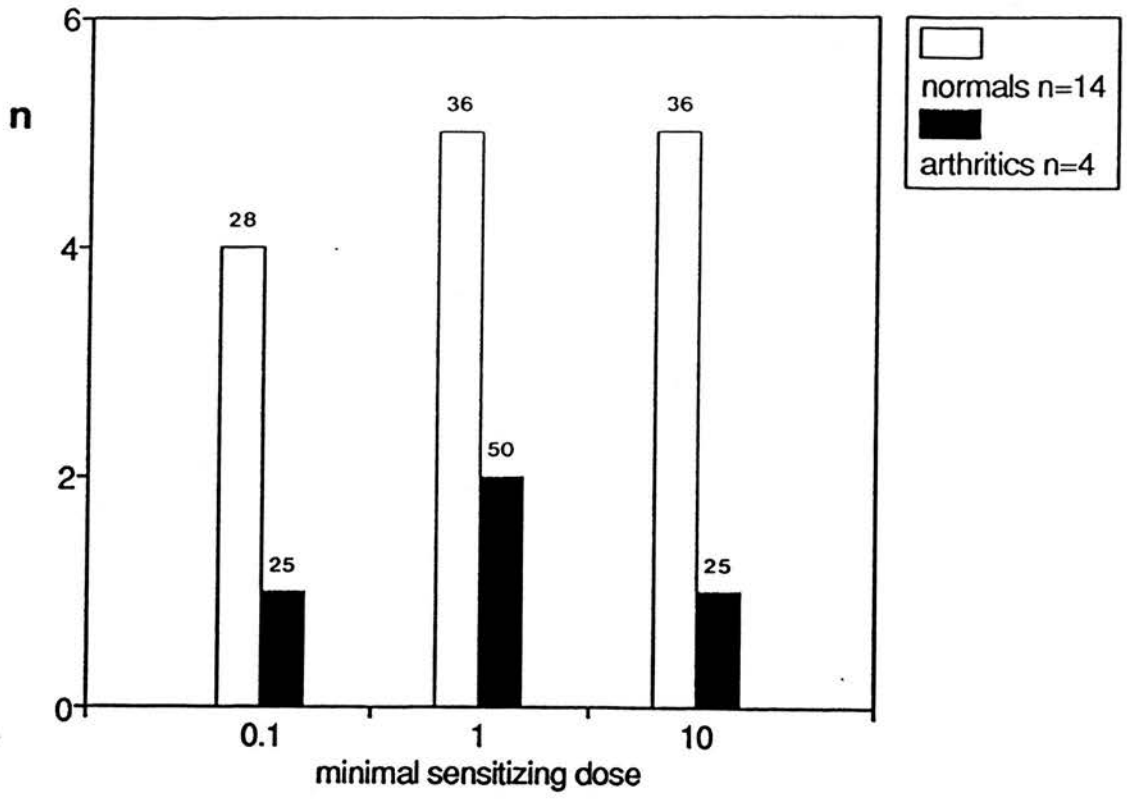


Fig. 5.4 Minimal effective doses of bradykinin causing increased responsiveness of high threshold mechanoreceptors to mechanical stimulation in normal (n=14) and arthritic (n=4) joints. The number of units (n) responding to minimal effective doses of 0.1, 1.0 and 10 μg are shown. The percentage of excitable units giving an initial response to each dose is given above the appropriate bar. No clear difference in minimal effective dose was seen between units from normal and arthritic joints.

nine units a reduction in sensitization response was observed with increasing doses of bradykinin (Fig 5.6). There was no correlation between units that were excited and those that were sensitized to mechanical stimuli in terms of dose-related effects. Thus, three units which showed reduced responsiveness to mechanical sensitization were excited in a dose-dependent manner, and four units which were sensitized by bradykinin in a dose-dependent manner were susceptible to reduced excitation at higher doses. For doses of 0.1 - 10 μg, maximal unit responses were produced by the full range of doses (Fig. 5. 8).

Arthritic joints

Nine mechanonociceptors were examined for the effects of bradykinin (0.1 - 40 μg, i.a.) on their responsiveness to mechanical stimuli. Of these units, four (44%) displayed an increased responsiveness to mechanical stimuli following injection of bradykinin. The minimal dose for receptor sensitization was between 0.1 and 5 μg (fig. 5.4). Bradykinin-induced changes in responsiveness had a mean latency of between 15 seconds and 4 minutes (mean: 2 ± 1 mins), and lasted for between three and ten stimuli (mean duration: 4.5 ± 0.4 mins).

Of four units which were examined for dose-dependent effects, only two responded in a dose-dependent manner (Fig 5.7). For the other two units, desensitization to increasing doses of bradykinin occurred. For doses of 0.1 - 10 μg, maximal unit responses to injections of bradykinin were produced by the full range of doses (Fig. 5.8).

Fig. 5.5 Dose-dependent effects of increased responsiveness of articular mechanonociceptors caused by bradykinin in normal joints. Each bar represents the mean response of four units to mechanical stimuli applied every two minutes following injection of bradykinin (upper panel, 0.1 μg; middle panel, 1.0 μg; lower panel, 10 μg, i.a.). Significant increases were only seen with the highest dose used of 10 μg. * $p < 0.05$.

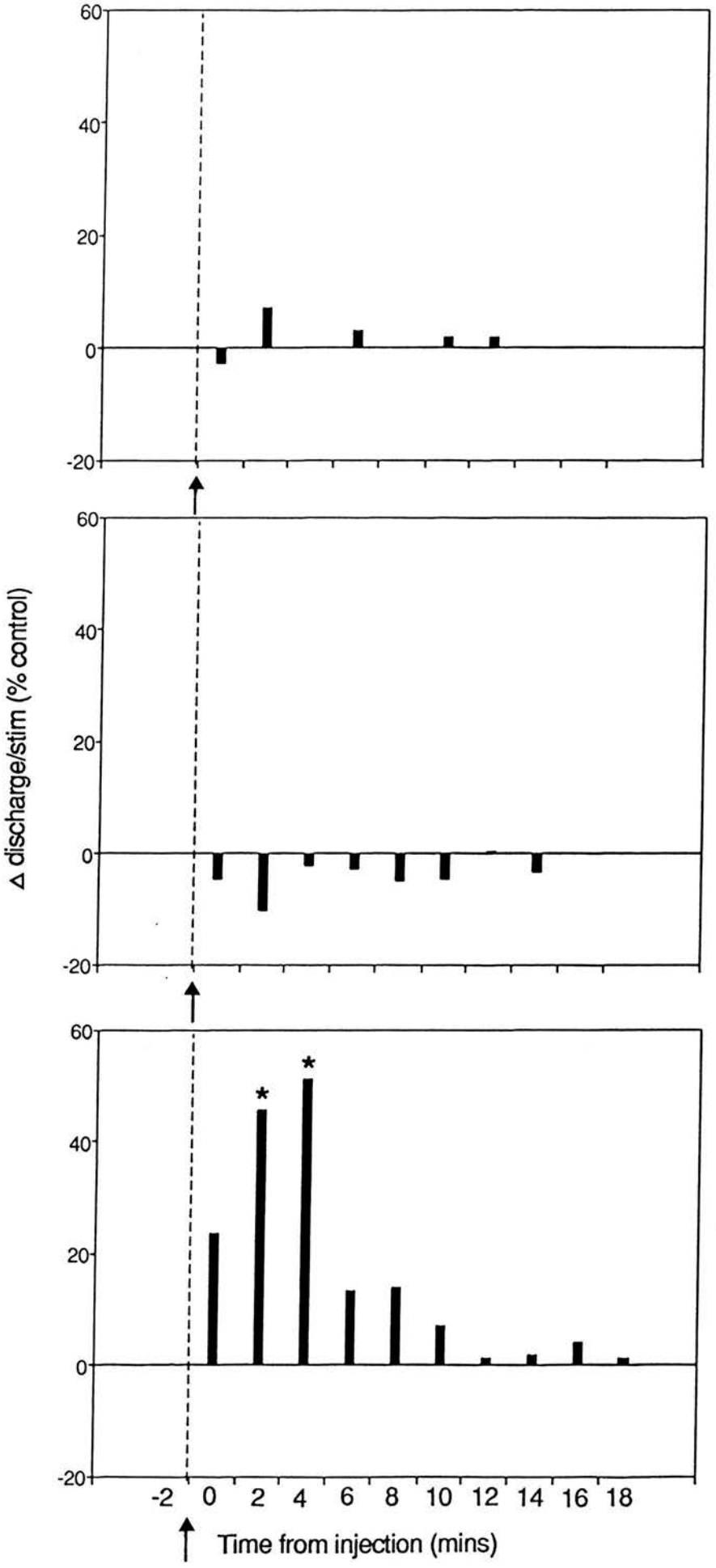
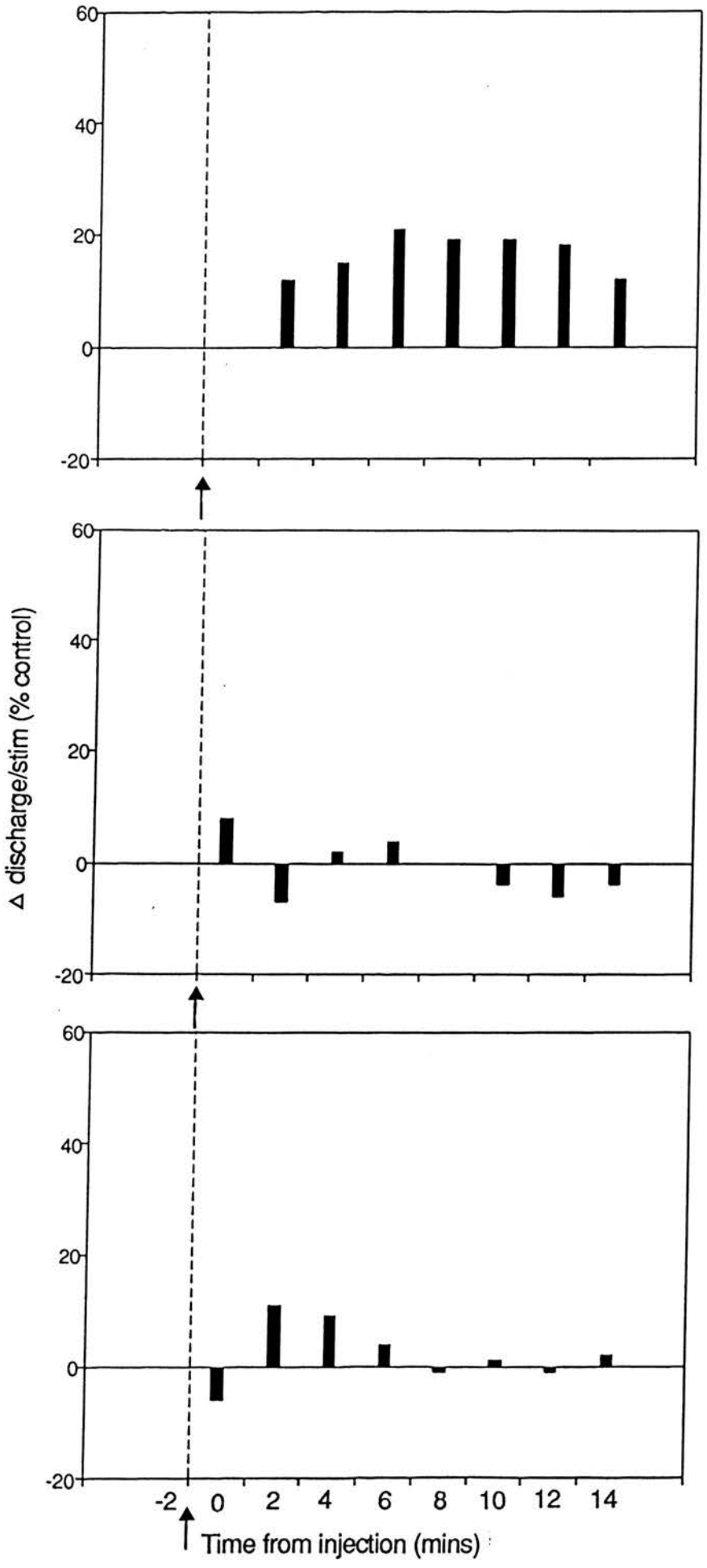

60
40
20
0
-20
60
40
20
0
-20
60
40
20
0
-20
*
*
Δ discharge/stim (% control)
-2
0
2
4
6
8
10
12
14
16
18
Time from injection (mins)

Fig. 5.6 Desensitization of increased mechanonociceptor responsiveness to increasing doses of bradykinin. Each bar represents the mean response of nine units to mechanical stimuli applied every 2 minutes following injection of bradykinin (upper panel: 0.1 μg, middle panel: 1.0 μg, lower panel: 10 μg, i.a.).


60
40
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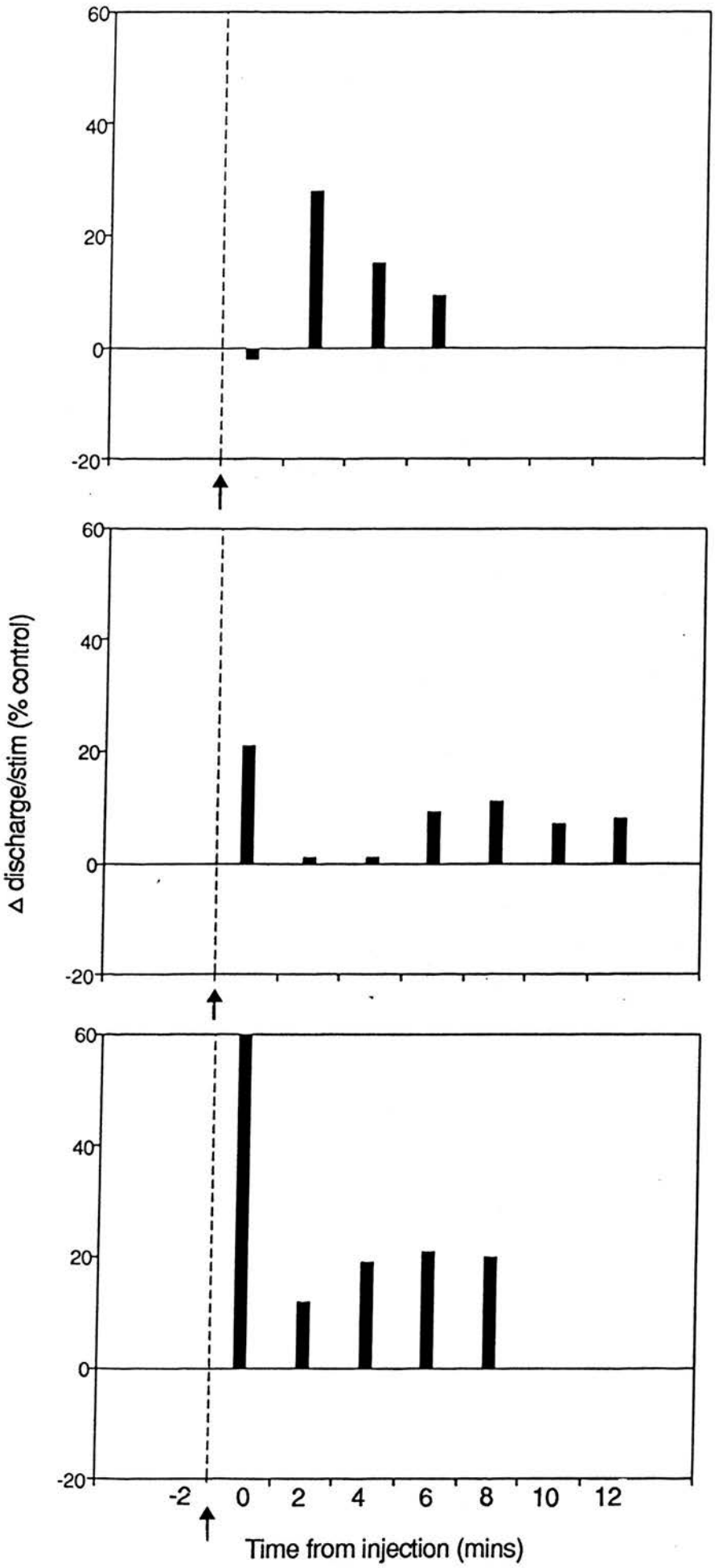
-20
60
40
20
0
-20
60
40
20
0
-20
Δ discharge/stim (% control)
-2
0
2
4
6
8
10
12
14
Time from injection (mins)

Fig. 5.7 Dose-dependent effects of bradykinin on increased responsiveness of mechanonociceptors in arthritic joints. Each bar represents the mean response of two units to mechanical stimuli applied at each time point following i.a. injection of bradykinin (upper panel: 0.1 μg, middle panel: 1.0 μg, lower panel: 10 μg)


60
40
20
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0
-20
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-20
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2
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10
12
Δ discharge/stim (% control)
Time from injection (mins)

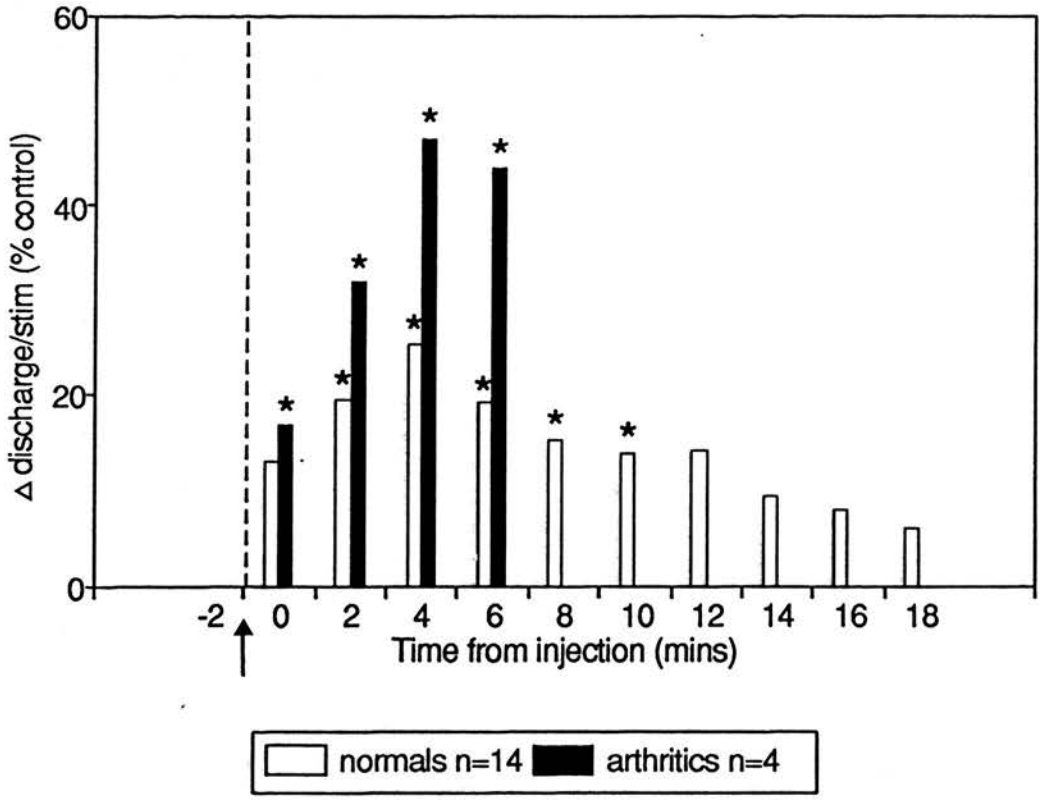


Fig. 5.8 Maximal bradykinin-induced increases in mechanonociceptor responsiveness in normal and arthritic rats. Maximal unit responses to bradykinin are illustrated, with each bar representing the mean value for responses to mechanical stimuli applied every 2 minutes. Maximal responses were caused by a median dose of 1.0 μg both in normals and arthritics. Although the peak effect was greater for units from arthritic animals, the response was of longer duration in normals. * $p < 0.05$.

5.2.2 Electrophysiology in vitro

Responses of articular sensory receptors to bradykinin were examined using the in vitro isolated hind limb preparation described in detail in the Section II. In eleven experiments afferent activity was examined both for units with identified mechanoreceptive fields in the ankle joint tissues, and units for which no response to mechanical probing was obtained, but which were activated by injection of bradykinin. All the units studied had action potential spike shapes similar to those of identified C fibre afferent units. Identified high threshold slowly adapting mechanoreceptors had afferent fibre conduction velocities ranging from 0.4 - 2.0 ms^{-1}.

5.2.2.1 Mechanonocieceptors

Injections of bradykinin (0.1 - 10 μg, i.a.) into the hind-limb perfusate produced excitation of only one mechanonociceceptor unit out of six that were excited by injections of capsaicin or other chemicals (see Section IV). The highest dose of 10 μg bradykinin was required to excite this receptor.

5.2.2.2 Chemosensitive units

Injections of bradykinin (0.1 - 10 μg) excited eight (89%) from nine non-mechanoresponsive units. The minimal effective dose for was between 0.1 and 10 μg, and for the single mechanosensitive unit was 10 μg (fig. 5.9). The mean latency to onset of the response to bradykinin was 36 ±

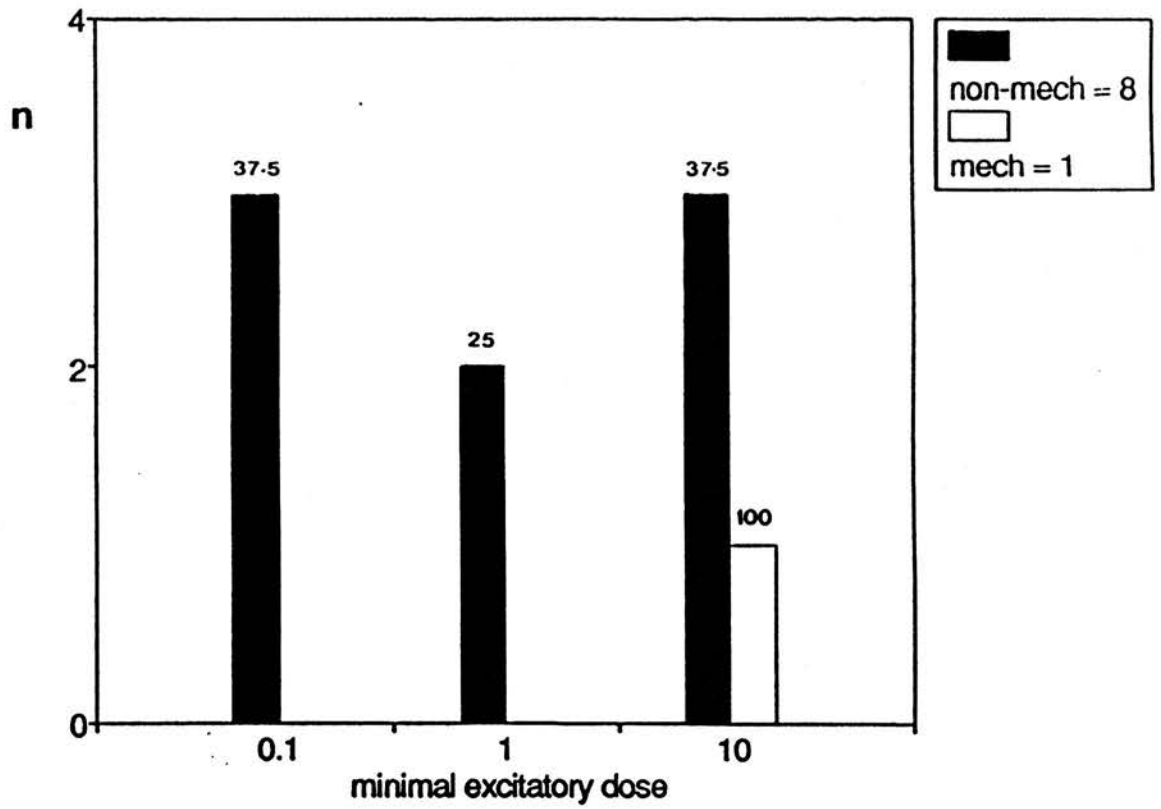


Fig. 5.9 Minimal effective doses for bradykinin-induced excitation of high threshold mechanoreceptor and non-mechanoresponsive units. Mechanoreceptors were relatively insensitive to the excitatory effects of bradykinin.

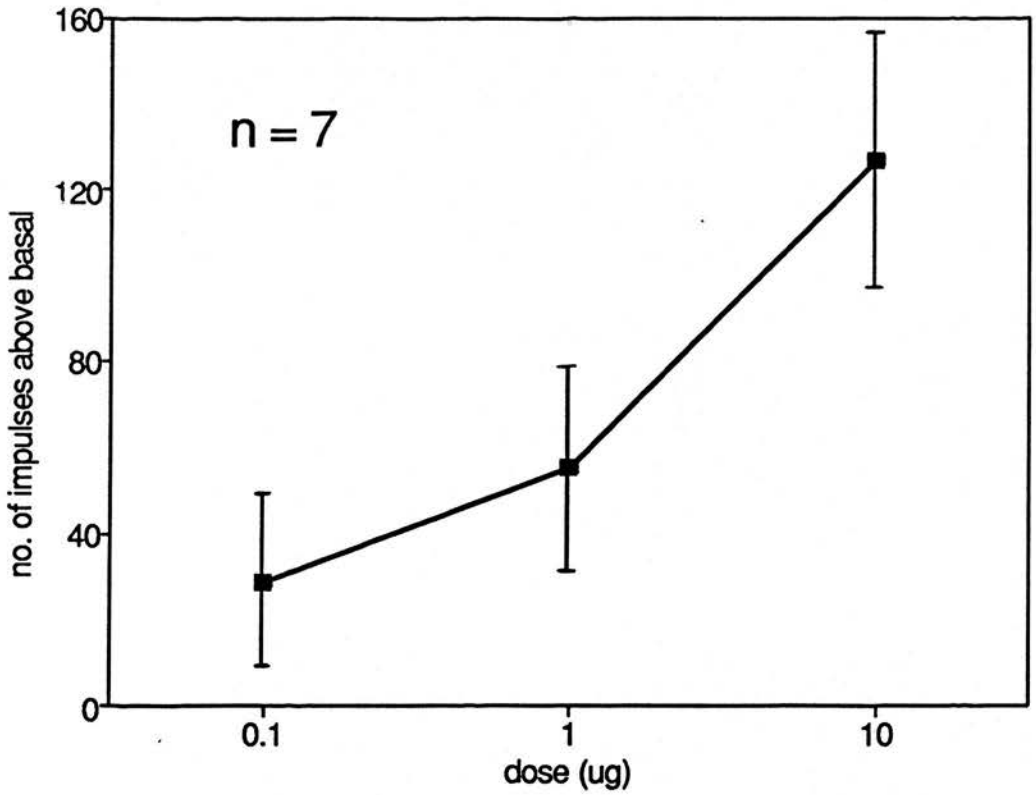


Fig. 5.10 Dose-dependent excitatory effects of bradykinin on articular sensory receptors. Dose-dependent responses were obtained in seven of nine units.

11 seconds, and the mean duration was 228 ± 30 seconds.

Dose-dependent effects of bradykinin were examined in all units for doses of 0.1 - 10 μg. From the nine non-mechanosensitive units seven responded in a dose-dependent manner, with the remaining unit displaying a reduced response to the highest dose used (fig. 5.10).

5.3 DISCUSSION

In this series of experiments intra-arterial injection of bradykinin both excited and increased the mechanical responsiveness of articular high-threshold mechanoreceptors with afferent fibre conduction velocities in the C and A-delta range. These responses are seen in both from normal and arthritic joints. Furthermore, demonstration of the excitatory effects of bradykinin on articular receptors has also been accomplished *in vitro*.

5.3.1 In vivo electrophysiology

5.3.1.1 Bradykinin-induced excitation

The doses of bradykinin (i.a.) required for receptor excitation in the present investigation were in the same order of magnitude as those shown to excite C or A-delta afferent units from the knee joint of the cat (Kanaka et al., 1985). In other feline tissues including muscle (Franz & Mense, 1975), viscera (Haupt et al., 1983) and skin (Beck & Handwerker, 1974) similar doses caused nociceptor excitation following intra-

arterial bolus injection. Over more prolonged periods of application, comparable concentrations of bradykinin have been shown to excite canine muscular (Kumazawa & Mizumura, 1977a) and testicular (Kumazawa & Mizumura, 1980b) polymodal nociceptors. Bradykinin has also been reported to excite cutaneous low-threshold slowly adapting mechanoreceptors (Beck & Handwerker, 1974; Fjallbrant & Iggo, 1961).

In the present study, no separation of effects on articular receptors with A-delta or C fibre afferents was found. This lack of selectivity has also been demonstrated for nociceptors from the cat knee joint (Kanaka et al., 1985), and from muscle (Mense, 1977), where approximately equal numbers of A-delta and C fibre units are excited by bradykinin.

Tachyphylaxis of responses to bradykinin, found here in articular receptors has been reported for nociceptors in many other tissues, and is a characteristic of bradykinin-evoked pain in the blister base (Keele & Armstrong, 1964). As in the present study, dose-response curves constructed for the effects of bradykinin on nociceptors from muscle (Mense, 1977) were often bell-shaped, or responses were of an all-or-nothing type.

Bradykinin excited 76% of units examined from the rat ankle joint, a smaller proportion than that found in studies on cat knee joint receptors (Kanaka et al., 1985) where thirty seven (92.5%) of forty C and A-delta afferent units were excited by bradykinin. In other tissues, similar proportions of responding units to those found in the present experiments have been reported. These include skin 52% (Beck & Handwerker, 1974), viscera 80% (Haupt et al., 1983), and muscle 60% (Mense, 1977; Franz & Mense, 1975). In two studies carried out on

cutaneous afferents, bradykinin did not produce any significant activation of C fibre afferents (Chahl & Iggo, 1977; Fjallbrant & Iggo, 1961).

Differences in the proportions of sensitive sensory receptors may reflect variation in tissue types, or heterogeneity in sensory receptor populations. Kanaka et al. (1985) favour the idea that poor access to particular receptors via the blood supply is responsible for this variation, and in their study only those units initially excited by i.a. KCl were included in their final analysis. In the present study injection of capsaicin (1 - 10 μg) at the end of the experiment excited two units which were not previously excited by bradykinin (see Section IV on capsaicin for further details). Desensitization of sensory units to bradykinin may also complicate the matter. A further source of variability arises from the demonstration that the algesic (Ferreira et al., 1973; Moncada et al., 1975) or excitatory (Chahl & Iggo, 1977) effects of bradykinin are dependent on the presence of prostanoids. It has in fact been shown that bradykinin-induced excitation of nociceptors from muscle or viscera is reduced by the administration of aspirin or indomethacin (Mense, 1982; Kumazawa et al. 1987). It follows that the presence of differing levels of prostanoids in tissues surrounding the receptors under study will produce changes in excitability (also see Section VII).

Direct excitatory effects of bradykinin on dissociated or cultured sensory neurones have recently been demonstrated (Higashi et al., 1982; Baccaglini & Hogan, 1983; Weinreich, 1986; Lindsay & Rang, 1987) and provide information regarding the mechanisms by which bradykinin induces nociceptor activation. Bradykinin causes an increased sodium conductance

in the soma of sensory neurones (Burgess et al., 1989). This is associated with activation of phospholipase C, which results in the generation of the second messengers 1,4,5-inositol triphosphate and diacylglycerol. Diacylglycerol is known to activate protein kinase C, and studies with phorbol esters and protein kinase C inhibitors suggest that this enzyme is important in generating the excitatory inward current (Burgess et al., 1989). Although the actions of bradykinin are strongly dependent on the presence of other mediators in vivo, these findings provide evidence that bradykinin is capable of producing excitatory effects of its own.

5.3.1.2 Mechanonociceptor sensitization

In the present series of experiments, in addition to activating articular mechanononociceptors, bradykinin also increased their responsiveness (sensitization) to mechanical stimuli. Bradykinin-induced sensitization of nociceptors to mechanical stimuli has been reported previously in muscle (Mense & Meyer, 1987), where sensitization of 38% of C and 67% of A-delta nociceptive afferent units was produced following local intramuscular injection. As demonstrated in cat muscle (Mense & Meyer, 1987), sensitization of ankle joint receptors shown here can occur without activation, and vice versa. Furthermore, in the present study, the two effects show independent desensitization, suggesting that two different mechanisms may be responsible for bradykinin-evoked excitation and sensitization.

The mechanism of action of bradykinin induced sensitization may not involve a direct action on the nerves themselves, but may involve the

release of other mediators from surrounding tissues. As mentioned above bradykinin releases prostaglandins from many tissues (Juan et al., 1984) and therefore sensitization may be produced by the action of the prostanoids on the sensory receptors (Schaible & Schmidt, 1988b). Other substance are also released by bradykinin including substance P (Lembeck & Holzer, 1979) and leukotrienes (Samuelsson, 1983).

Results from electrophysiological studies on isolated sensory neurones suggest that bradykinin may increase nociceptor excitability by a direct effect of its own. As well as the protein kinase C mediated depolarization mentioned above (Burgess et al., 1989), bradykinin also inhibits a slow after-hyperpolarization in sensory neurones via blockade of Ca^{2+}-dependent (Weinreich, 1986). Inhibition of the slow after-hyperpolarization leads to an increased level of excitability and increases the potential firing rate in C-type nodose ganglion neurones of the rabbit (Weinreich & Wonderin, 1987). Application of forskolin mimics this effect of bradykinin, suggesting that it is mediated via cyclic AMP.

The identification of two distinct mechanisms by which bradykinin may excite or sensitize nociceptive neurones provides a possible explanation for the independent occurrence of bradykinin-induced excitation and sensitization in the present study. The involvement of separate second messenger systems in the two types of response could also explain the lack of cross desensitization seen here. Involvement of different bradykinin receptors is unlikely, since both the excitatory and sensitizing effects of bradykinin have been reported to be B2 receptor mediated (Mizumura et al., 1990; Rang & Heyman, 1990).

Actions of bradykinin in arthritic joints

The ability of bradykinin to excite and sensitize high-threshold articular mechanoreceptors suggests that endogenous bradykinin could play a role in the increased responsiveness of these receptors in chronically inflamed joints. However, the behaviour of C and A-delta afferent units in the arthritic joint (Guilbaud et al., 1985) differs from that produced by bradykinin in these experiments. Long-lasting increases in spontaneous activity were not seen following bradykinin administration, and receptor sensitization was generally short-lived.

In arthritic joints, bradykinin was less potent than in normal joints with regard to both excitation and sensitization of receptors. Repeated administration of bradykinin in arthritic joints resulted in a marked tachyphylaxis. During inflammation, when endogenous levels of bradykinin are likely to be elevated (Lewis, 1970), additional application of exogenous bradykinin will have to act on an elevated baseline. Thus large increases will not be produced and susceptibility to desensitization will be greater.

In addition to causing the release of other mediators involved in the production of inflammatory pain, bradykinin has also been shown to cause local release of opioid peptides (Kudo et al., 1986a,b). Several lines of evidence exist to suggest that opioids may exert a peripheral analgesic effect, particularly during inflammation (Ferreira & Nakamura, 1979b; Hargreaves et al., 1988; Stein et al., 1988). Opiate-induced reduction in afferent discharge from the inflamed cat knee joint has also been described (Russell et al., 1987). It is possible that in the inflamed joint, endogenous opioids may be partly responsible for the

variable responses produced by bradykinin in the present study.

Levels of bradykinin found in inflammatory exudates are generally low and in man there is no correlation between pain severity and kinin concentration in chronic arthritis (Melmon et al., 1967). These findings, together with the results described above, support the idea that bradykinin is involved in the production of pain when released acutely. However, suseptibility to tachyphylaxis, and its inability to cause sustained increases in afferent discharge and nociceptor excitability, suggest that bradykinin could not be solely responsible for the changes in nociceptor behaviour seen in chronically inflamed rat ankle joints. Further investigations using selective bradykinin receptor antagonists may help to determine the role played by the peptide in enhanced nociceptor activity in arthritic joints.

5.3.2 In vitro electrophysiology

The demonstration that bradykinin excites articular receptors *in vitro* suggests that this effect is not secondary to the systemic release of other blood-borne mediators or to spinal reflex mechanisms. In the present experiments, *in vitro* responses to bradykinin were dose-dependent, or displayed desensitization with increasing doses, and as such were essentially the same as those seen *in vivo*. Identified high threshold mechanoreceptors were not excited as effectively *in vitro*, suggesting that the responses of these units are dependent on certain conditions present *in vivo*. This difference in responsiveness may be partly due to the lower operating temperature *in vitro* (Kumazawa & Mizumura, 1983), or may be due to the perfusion fluid washing out other

substances present in the tissues, such as endogenous prostanoids. Indeed, results reported in Section VII of this thesis dealing with the potentiating effects of the prostanoids support this idea. The potent excitatory effect of bradykinin on non-mechanosensitive fine afferent units demonstrated in this study, has also been reported by other workers for cutaneous receptors (Meyer & Campbell, 1988; Davies et al., 1989).

SECTION VI

EFFECTS OF 5-HT ON ARTICULAR SENSORY RECEPTORS IN THE RAT ANKLE JOINT

SECTION VI

EFFECTS OF 5-HT ON ARTICULAR SENSORY RECEPTORS IN THE RAT ANKLE JOINT

6.1 INTRODUCTION

Keele & Armstrong (1964) demonstrated that 5-HT has the ability to cause pain when applied to a blister base in man, and 5-HT was later shown to lower thresholds for chemically-induced pain in humans (Sicuteri et al., 1965), and to enhance pseudoaffective responses to bradykinin in animals (Nakano & Tiara, 1976). Sensory nerve endings associated with small myelinated and non-myelinated axons are activated and sensitized by 5-HT in skin (Fjallbrant & Iggo, 1961; Beck & Handwerker, 1974) and muscle (Mense, 1981), as are cutaneous slowly adapting type II mechanoreceptors with rapidly conducting afferent fibres (Fjallbrant & Iggo, 1961).

Although the pain produced by application of 5-HT to a blister base is antagonized by ICS 205-930, and therefore probably involves a 5-HT_3 receptor (Donatsch et al., 1984b; Richardson et al., 1985), in most cases the pharmacological identity of the 5-HT receptor associated with nociceptive sensory endings has not been established. The present study was undertaken to examine the effects of 5-HT on nociceptors in the rat ankle joint, and to characterize the 5-HT receptors mediating these effects by the use of selective receptor antagonists (Fozard et al., 1984; Bradley et al., 1986; Brittain et al., 1987; Butler et al., 1988).

Rats with localized adjuvant arthritis were used to investigate whether 5-HT plays a role in the increased sensitivity of articular mechanonociceptors in chronically inflamed arthritic joints.

6.2 RESULTS

Two types of afferent unit activity were investigated: mechano-nociceptors for which receptive fields were identified in the joint capsule; and chemosensitive (see Section IV) afferent units which either had an ongoing discharge or were excited by 5-HT, but had no identifiable receptive fields for mechanical stimuli (Table 6.1).

6.2.1 Electrophysiology in vivo

6.2.1.1 Mechanonociceptors

Normal joints

Mechanosensitive units with with receptors in normal ankle joint capsular tissues were high-threshold, and slowly adapting with punctate receptive fields of approximately 1mm diameter. Units had afferent fibre conduction velocities in the range 2.1 - 10.5 ms^{-1}. Only one mechanosensitive unit had an ongoing discharge (0.2 i.p.s.) before the addition of 5-HT, the rest were not spontaneously active.

Arthritic joints

On average approximately three times as many mechanonociceptors were found in athritic ankle joints compared with normals. The conduction velocities of their afferent fibres ranged from 0.5 - 7.8 ms^{-1}. In contrast to the lack of ongoing mechanonociceptor activity in normal rats nine units had an ongoing low level of discharge equal to 1.2 ± 0.35 i.p.s. before the administration of 5-HT.

6.2.1.2 Effects of 5-HT on responsiveness to mechanical stimuli

Normal joints

In the six units examined, injection of 5-HT (1-100 μg, i.a) produced a dose-dependent increase in responsiveness (sensitization) to the standard mechanical stimulus (fig. 6.1). The minimal effective dose was found to be 5 μg, this dose giving reproducible responses in all of the units examined. As illustrated in figure 6.3 a mean peak increase of 56% (n=4) was observed in response to subsequent stimuli following injection of a minimally effective dose of 5μg 5-HT, and this effect lasted for 1 to 2 mechanical stimuli. Individual unit responses are illustrated in figure 6.2. Larger doses of 5-HT had a more prolonged action as can be seen in figure 6.1, where the response to a mechanical stimulus was still elevated three minutes after the injection of 100 μg. Repeated injections of 5-HT produced a sensitization to the drug in four mechanonociceptors.

Fig. 6.1 Effects of 5-HT on the activity of a mechanonociceptor from a normal animal. (A) Neurogram showing the discharge pattern of the fast response evoked by i.a. injection of 100 μg 5-HT at the arrow. (B) Neurogram showing the discharge pattern of the slow response for the same injection of 5-HT (at arrow) using a slower sweep speed. The line trace below each neurogram represents the application of the mechanical stimulus. (C) Computer-generated plot illustrating the fast and slow responses to the same injection of 5-HT (at arrow) as shown in the neurograms. Mechanical stimuli (arrowheads) were repeated once every minute, and it can be seen that mechanical responsiveness was increased following the injection. The number of action potentials evoked per stimulus is given above each response. The inset shows 100 superimposed triggered oscilloscope traces of the single afferent unit whose discharge was counted. This unit had an afferent fibre conduction velocity of 4 ms^{-1}.

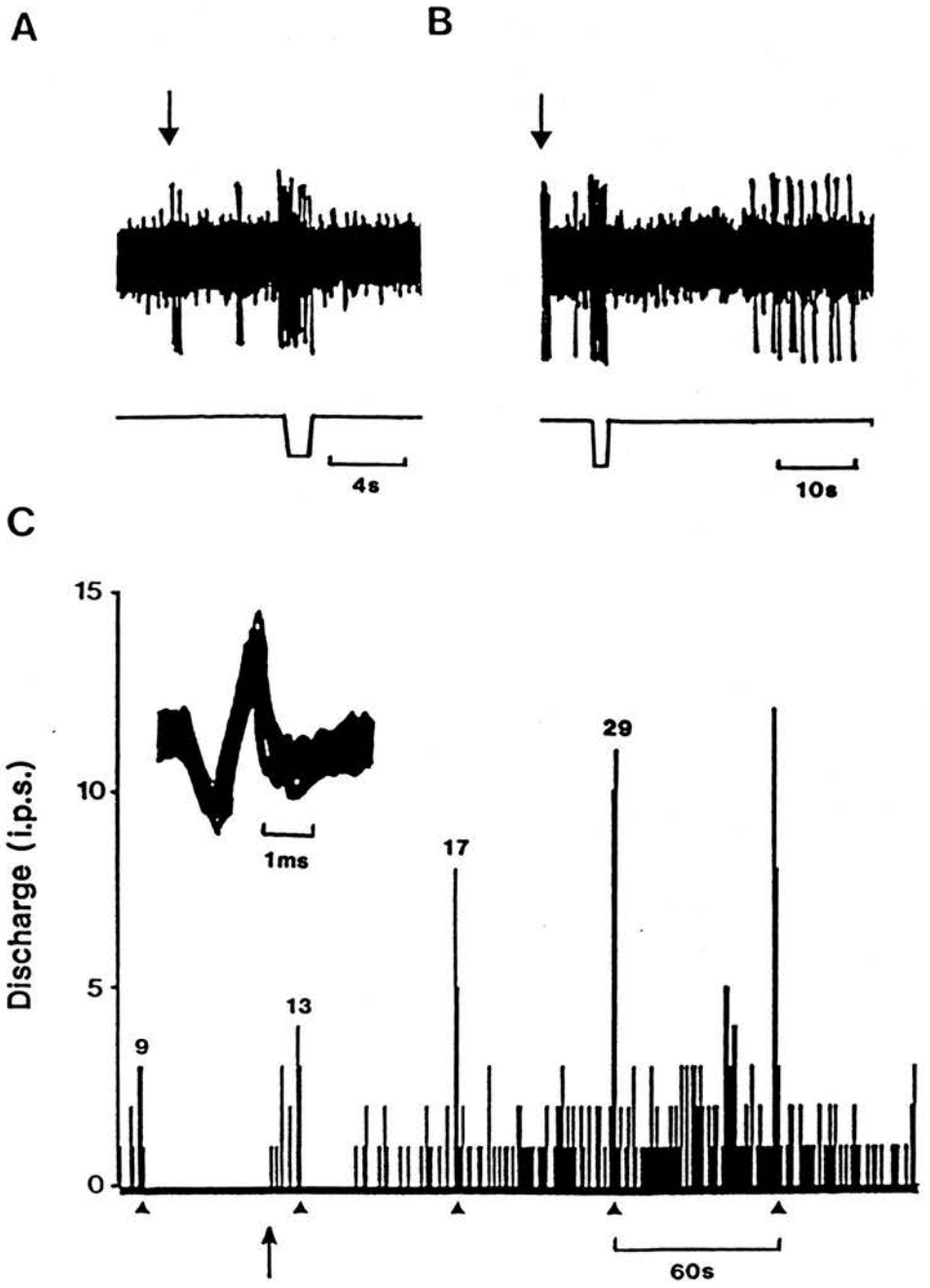

A
B
4s
10s
C
15
10
5
0
Discharge (i.p.s.)
1ms
9
13
17
29
60s

Fig 6.2 Summary of the effects of 5-HT on responsiveness to mechanical stimuli in mechanonociceptors from normal and arthritic joints Each bar represents the response of a single mechanoreceptor to a mechanical stimulus of 2 s duration repeated once every 2 mins both before and after injection of 5-HT (at arrow). Values are expressed as a percentage of the pre-injection (-2 min) response. Mechanonociceptor responses are shown for i.a. injection of 5 μg 5-HT in four normal joints, and 1 μg 5-HT in six arthritic joints. Although the time to peak and duration of the sensitization evoked by 5-HT differed between units, it can be seen that both the peak responsiveness and duration of effect were greater in units from arthritic joints than those from normal joints.

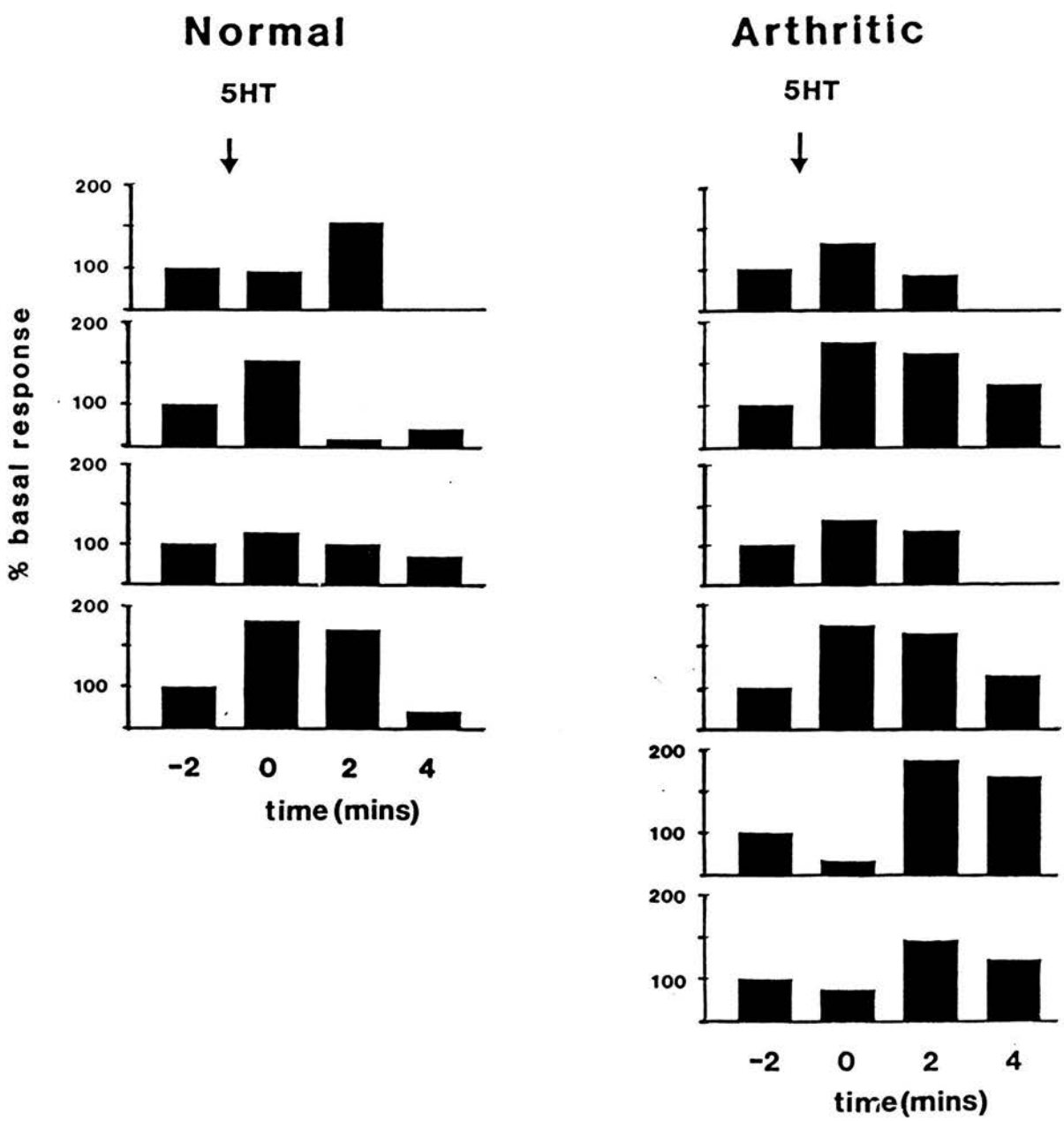
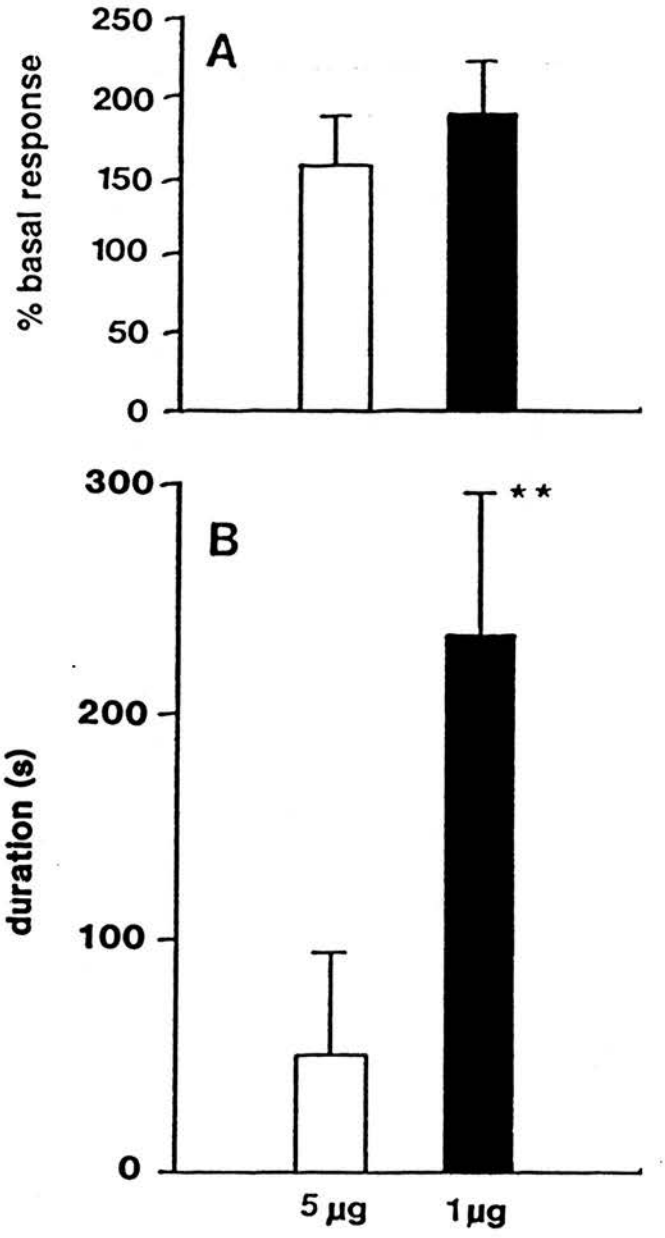

Normal
5HT
200
100
200
100
200
100
200
100
% basal response
-2
0
2
4
time (mins)
Arthritic
5HT
200
100
200
100
-2
0
2
4
time (mins)

Fig. 6.3 Effects of 5-HT on mechanoreceptor responsiveness both in normal and arthritic joints. (A) Bar graph showing the mean increase in mechanonociceptor responsiveness at the peak of the response evoked by 5 μg 5-HT (i.a.) in four normal joints (open bar), and by 1 μg 5-HT in six arthritic joints (filled bar). Values are given as a percentage of the pre-injection response. (B) Bar graph illustrating the mean duration of increased responsiveness produced by the same injection of 5-HT (normal joints, open bar; arthritic joints, filled bar). Error bars represent s.e. mean. Significantly different values are shown as **, when $p < 0.01$ (Wilcoxon).


A
% basal response
250
200
150
100
50
0
B
duration (s)
300
200
100
0
**
5 µg
1 µg

Arthritic joints

In all ten units examined a dose-dependent increase in responsiveness to the standard mechanical stimulus was produced following the injection of 5-HT (1-100 μg). In six of these units the minimal effective dose for production of consistent responses was determined and found to be 1 μg. A mean increase of 75% (n=6) in response to subsequent mechanical stimuli was produced following injection of this minimally effective dose. This lasted for 1 to 3 mechanical stimuli, a duration which is significantly greater ($p < 0.05$, Wilcoxon) than that produced by the minimally effective dose of 5 μg 5-HT in normal rats (fig. 6.2). Individual unit responses are illustrated in figure 6.2. Sensitization of mechanonociceptor responses to 5-HT was observed in two units.

6.2.1.3 Excitatory effects of 5-HT on mechanonociceptors

Normal joints

From six mechanonociceptors, i.a. injection of 1 - 100 μg 5-HT evoked a discharge in three previously silent mechanonociceptors and increased the discharge of one unit from a very low initial level of discharge. Thus 66% of the units were excited by 5-HT. The effect was reproducible in two of these units at the highest dose used (100 μg 5-HT). A two-component response was observed following injection of 5-HT consisting of transient burst of activity with rapid onset (<10 s), followed by a delayed (>20 s), longer lasting increase in discharge (fig 6.1). A summary of responses evoked by injections of 5-HT can be seen in table 6.1, together with data from arthritc joints.

Arthritic joints

Increases in spontaneous activity of six (60%) of ten mechanonociceptors was seen following injection of 5-HT (1 - 100 μg, i.a.). The biphasic response seen in normal animals was much less conspicuous, being obtained in only one recording. One unit gave only the fast response, and three units responded with only the delayed increase in spontaneous activity. Using counting periods of 15 s duration, in four units the slow response consisted of a mean increase of 1.6 ± 0.6 i.p.s. above basal discharge (1.1 ± 0.7 i.p.s) at the peak of the response. This response lasted for 185 ± 34 seconds. This effect of 5-HT contrasts markedly with the small response obtained following injection of 100 μg 5-HT in the normal rat (fig. 6.4). Ongoing activity in a seventh unit showed only a depression of activity following 5-HT administration.

6.2.1.4 Chemosensitive units

Normal joints

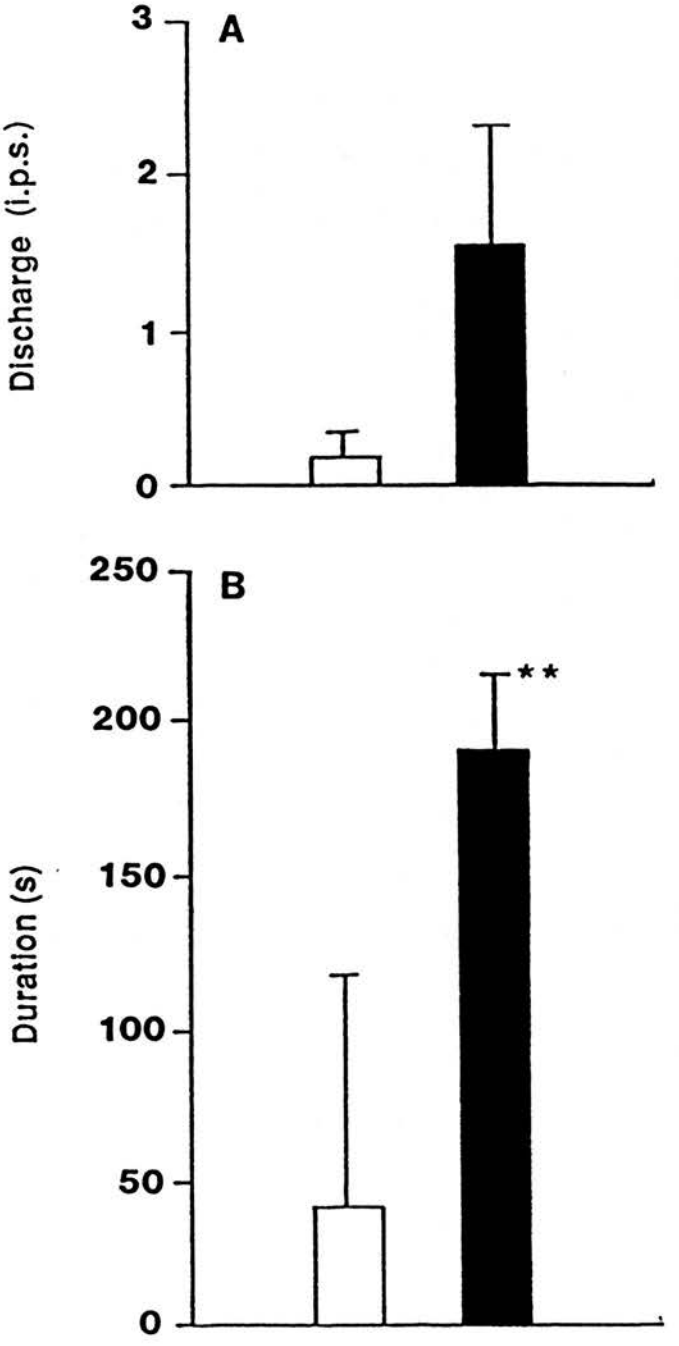
In the twelve normal animals examined in this study sixteen recordings consisting of between one and three different action potentials were obtained from units with a low level of activity before the addition of 5-HT. Their action potential spike shape characteristics were similar to those of identified C fibre afferents (see Section III), and their mean rate of discharge was 1.4 ± 0.3 i.p.s.

Table 6.1 Summary of the number of mechanonociceptors and chemosensitive units excited by 5-HT and the minimal effective doses for these effects.

	unit type	no. of units	no. of units displaying each type of response		min. effective dose (μg)
			rapid	delayed	
NORMAL	mechanonociceptor	6	4 (67%)	3 (50%)	100
	chemosensitive	16	12 (75%)	12 (75%)	1
ARTHRITIC	mechanonociceptor	10	3 (30%)	5 (50%)	1
	chemosensitive	12	7 (58%)	12 (100%)	1

The percentage of units responding with each type of response is shown in brackets

Fig. 6.4 Comparison of 5-HT-induced slow excitation of mechanosensitive units in normal and arthritic rats. (A) Bar graph showing the peak increase in discharge produced by 100 μg 5-HT (i.a.) in two responding units of six tested from normal rats (open bars), and the mean increase produced by 1 μg 5-HT in four of five units from arthritic ankle joints (filled bar). The mean basal rate of discharge in arthritic rats was 1.1 ± 0.8 i.p.s., whereas in control rats no activity was present before the injection of 5-HT. (B) Bar graph showing the duration of effects for the same injections shown in A. The vertical lines above each bar represent the s.e.m. for each value.


A
3
2
1
0
Discharge (i.p.s.)
B
250
200
150
100
50
0
Duration (s)
**

Arthritic joints

Spontaneously active units without identifiable mechanosensitive receptive fields were more numerous in arthritic rats than in normals. In experiments on ten animals the effects of 5-HT on spontaneous discharge were examined in twelve recordings consisting of between one and three different action potentials from units with spike shape characteristics similar to those of identified C fibre afferents. Their mean rate of spontaneous discharge before the administration of 5-HT was 1.4 ± 0.2 i.p.s.

6.2.1.5 Excitatory effects of 5-HT on chemosensitive units

Normal joints

All the units with an ongoing discharge were excited by an initial or subsequent injection of 5-HT (1-100 μg, i.a.); a further three units became active following the administration of the drug. Following the failure of initial doses of 5-HT to evoke a response, activation of individual units by 5-HT was seen with subsequent injections.

Two main components could be recognised in the response to 5-HT. An early, brief burst of activity, which was seen in 75% of active units followed by a sustained increase in background afferent discharge in 75% of units. Responses in individual units were either monophasic or biphasic; fast responses occurred within 10 seconds following injection of 5-HT and lasted for a maximum of 30 seconds. Desensitization developed to repeated injections of 5-HT (20-100 μg) at 10 minute

intervals. However, with lower doses (1-10 μg), and a 15 minute interval between injections, a relatively consistent response was obtained in four recordings. The slow response generally took longer than 15 seconds to develop and lasted for on average 105 ± 11 seconds. Depression of activity following the initial excitation was also seen in a small number of units when background activity was elevated.

Arthritic joints

All of the units examined were responsive to injections of 5-HT (1-100 μg). A two component response was seen as normal animals. A fast excitatory response was seen in 58% of units, and in all the units studied a delayed, long-lasting (121 ± 17 seconds) increase in discharge was observed.

6.2.1.6 Effects of 5-HT receptor antagonists in normal and arthritic joints

The 5-HT receptor antagonists MDL 72222, ICS 205-930 and ketanserin were administered at 100 μgkg^{-1}, i.a., doses previously found to be active in abolishing chemoreceptor responses to 5-HT in the cat (Kirby and McQueen, 1984). The $5\text{-}HT_3$ receptor antagonists MDL 72222 and ICS 205-930 are structurally related to cocaine (Richardson et al., 1985), and may possess some local anaesthetic properties. While GR38032F has been shown to be highly selective and specific in its antagonism of $5\text{-}HT_3$ receptors when administered <u>in vitro</u> at doses up to 1 $mgkg^{-1}$ (Butler et al., 1988), the $5\text{-}HT_2$ receptor antagonist ketanserin may produce hypotension

via antagonist activity at α-adrenergic receptors (Vanhoutte, 1985). Evidence for a selective and specific effect of the antagonists used in the present experiments is provided by the observation that the 5-HT_3 receptor antagonists selectively blocked the 5-HT_3 receptor mediated Bezold-Jarisch-like reflex (bradycardia) evoked by 5-HT, and ketanserin selectively antagonized a 5-HT-induced hypotension. Furthermore, capsaicin-induced excitation (see Section IV) was not blocked by either the 5-HT_3 or 5-HT_2 antagonists.

Mechanonociceptor responsiveness

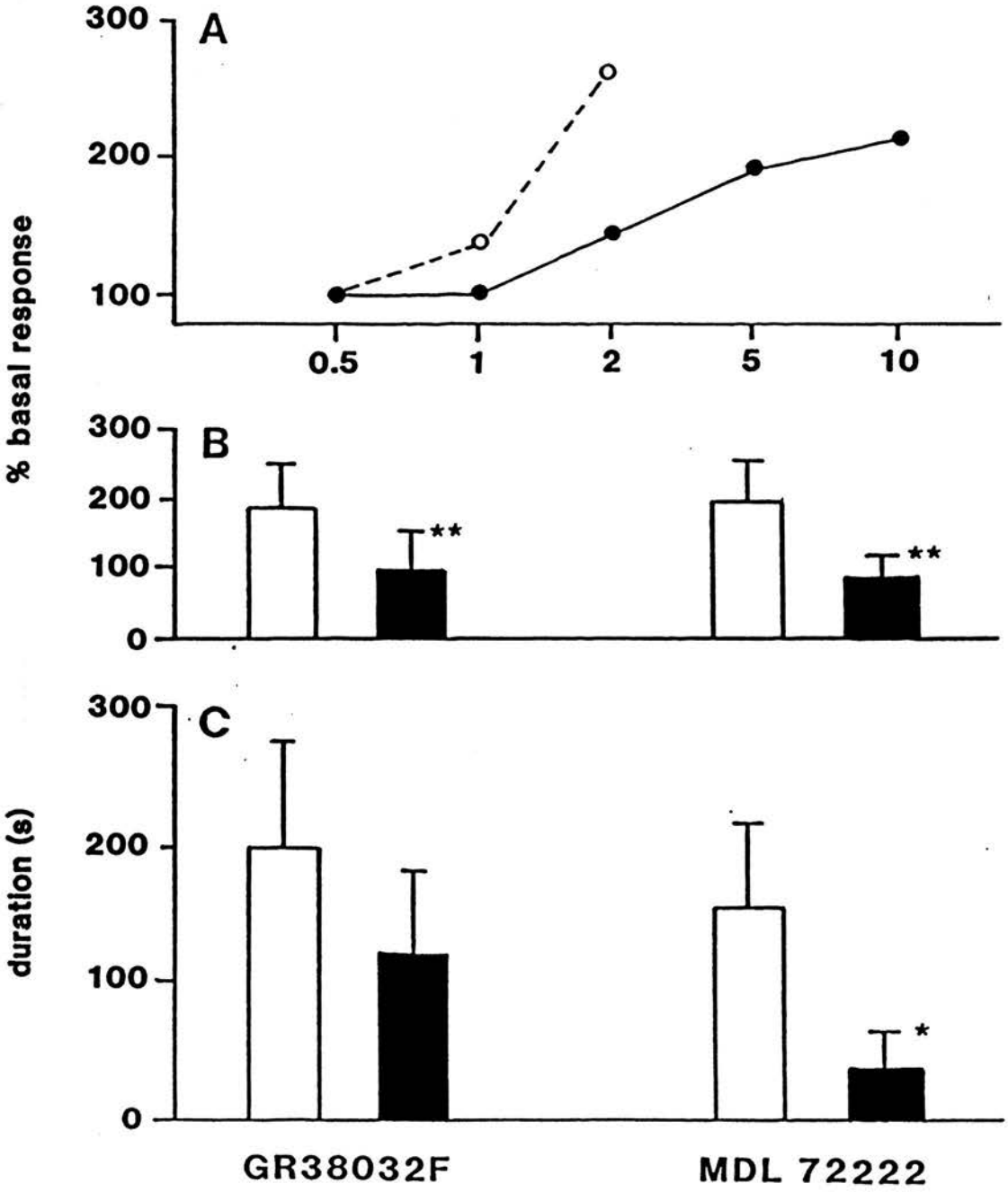
The 5-HT_3 receptor antagonists MDL 72222, ICS 205-930 and GR38032F administered intra-arterially, each antagonized the 5-HT-induced increase in responsiveness of mechanonociceptors both in normal and arthritic joints. In five units treatment with MDL 72222 (100 μgkg^{-1}) markedly reduced the increased responsiveness produced by 5-HT; injection of ICS 205-930 (100 μgkg^{-1}) produced a clear rightward shift in the 5-HT dose response curve in one unit; in studies on five units, GR38032F (100 μgkg^{-1}) abolished the response in one unit and produced a marked reduction in the response in four units (see Fig.6.5).

The 5-HT2 receptor antagonist, ketanserin (100 μgkg^{-1}), did not affect the 5-HT evoked increase in mechanonociceptor responsiveness, either in normal or arthritic joints when tested in six units.

Activity of mechanonociceptors

In spontaneously active mechanonociceptors from arthritic joints the 5-

Fig. 6.5 Effects of 5-HT_3 antagonists GR 38032F (100 μgkg^{-1}, i.a.), MDL 72222 (100 μgkg^{-1}, i.a.) or ICS 205-930 (100 μgkg^{-1}, i.a.) on 5-HT-induced enhancement of mechanonociceptor responsiveness both in normal and arthritic joints. (A) Graph showing a shift to the right in the log dose-response curve caused by ICS 205-930 (100 μgkg-1, i.a.) on a mechanoreceptor with afferent fibre conduction velocity of 2.7 ms-1 from an arthritic joint. Open circles and filled circles represent responses before and after addition of antagonist respectively. The dose of 5-HT is shown in μg with the peak response obtained given as a percentage of the pre-injection control response. (B) Bar graph illustrating the effect of GR38032F (n = 5) and MDL 72222 (n = 5) on the mean peak increase in mechanoreceptor responsiveness produced by an effective standard dose of 5-HT (1 - 100 μg, i.a.). Bars represent peak responses before (open) and after (filled) injection of antagonist. (C) Bar graph showing the duration of response obtained for the same injections as in A. Error bars represent s.e. mean. Significantly different values are shown as * when $p < 0.05$, and ** when $p < 0.01$ (Wilcoxon).


A
B
C
% basal response
duration (s)
300
200
100
0
0.5
1
2
5
10
**
**
*
GR38032F
MDL 72222

HT_3 antagonists MDL 72222 (100 μgkg^{-1}, n=1) or GR38032F (100 μgkg^{-1}, n=2) caused reductions in ongoing activity of 70% and 65% (range: 30 - 100%) respectively when injected on their own. Reductions of activity lasted for no longer than 5 minutes in each case. In two mechanosensitive units the 5-HT_2 receptor antagonist ketanserin (100 $\mu g kg^{-1}$) had no effect on spontaneous activity.

An examination of the effects of the various 5-HT antagonists on the two components of 5-HT induced increases in spontaneous activity was complicated by the inconsistent nature of the fast response and its marked susceptibility to desensitization. The slow response, however, was observed in all cases, and in arthritic animals neither MDL 72222 (100 μgkg^{-1}, n=1), ICS 205-930 (100 μgkg^{-1}, n=1) or GR38032F (100 μgkg^{-1}, n=1) had any affect on it. In one of two units ketanserin produced a marked shift to the right of the 5-HT dose response curve (Fig. 6.6).

Chemosensitive units

In the case of chemosensitive units the 5-HT_3 receptor antagonists MDL 72222, ICS 205-930 and GR38032F all reduced ongoing discharge in arthritic joints or in normal joints previously exposed to 5-HT (see table 6.2).. Reductions in activity produced by antagonists lasted for 1 - 3 minutes. The 5-HT_2 receptor antagonist ketanserin also markedly reduced ongoing discharge in arthritic and normal joints (table 6.2).

Analysis of the affects of the 5-HT receptor antagonists on the fast response to injection of 5-HT was complicated by inconsistency of the response and its susceptibility to desensitization. However, it was markedly reduced or abolished following injections of the 5-HT_3 receptor

Fig. 6.6 Effects of ketanserin (100 μgkg^{-1}, i.a.) on 5-HT-induced slow excitation of chemosensitive afferent units from normal and arthritic rats. (A) Bar graph showing the mean peak discharge, expressed as a percentage of pre-injection control, produced in response to a standard dose of 5-HT (1 - 100 μg, i.a.) before (open bar) and after (filled bar) injection of ketanserin (100 μgkg-1, i.a., n = 14). (B) Bar graph illustrating the mean duration of the effects shown in A. Error bars represent s.e. mean. Significantly different values are shown as ** when $p < 0.01$.

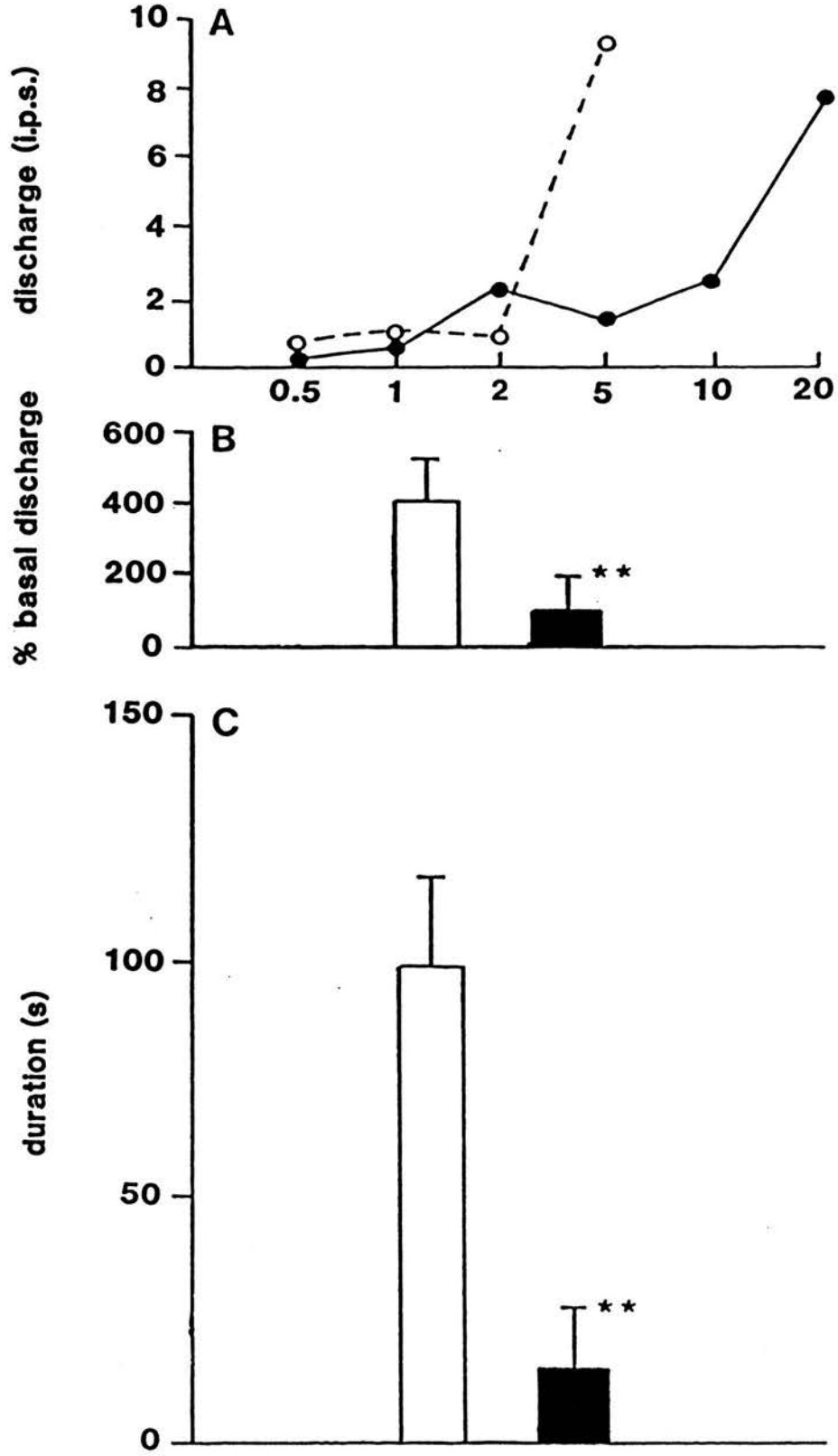

A
10
8
6
4
2
0
discharge (i.p.s.)
0.5
1
2
5
10
20
B
600
400
200
0
% basal discharge
**
C
150
100
50
0
duration (s)
**

Table 6.2 Summary of the effects of 5-HT antagonists on the spontaneous discharge of chemosensitive units

	MDL 72222		ICS 205-930		GR38032F		ketanserin	
	n	% reduction in discharge	n	% reduction in discharge	n	% reduction in discharge	n	% reduction in discharge
NORMAL	4/4	53 (43 - 68)	3/3	62 (33 - 100)	4/4	53 (64 - 99)	5/9	63 (43 - 68)
ARTHRITIC	1/4	100	0/0	-	4/4	36.5 (13 - 50)	6/9	58 (76 - 25)

Mean values were calculated only using responsive units - figures in brackets show the range of effect.
All antagonists were injected i.a. at a dose of 100 μgkg^{-1}

antagonists MDL 72222 (n=6), ICS 205-930 (n=4) or GR38032F (n=4) (all at 100 μgkg^{-1}, i.a.) (fig. 6.7), but occurred in three recordings following the administration of ketanserin (100 μgkg^{-1}, i.a.).

The slow response to injection of 5-HT was unaffected by the 5-HT_3 receptor antagonists, but was blocked or markedly reduced in fourteen of fifteen units by the 5-HT_2 antagonist ketanserin (100 μgkg^{-1}, i.a.)(Fig. 6.7).

6.2.2 Electrophysiology in vitro

Using the in vitro isolated hind limb preparation the effects of 5-HT were examined on mechanonociceptors and chemosensitive units with afferent axons in the PACR. Identified mechanonociceptor units had afferent fibre conduction velocities in the range 0.4 - 2 ms^{-1}, and chemosensitive units had action potential spike shape characteristics similar to those of identified C fibre units. Only normal joints were examined in this study.

6.2.2.1 Mechanonociceptor excitation

Six mechanonociceptors were examined for the effects of 5-HT (0.01 - 100 μg, i.a.). Three of these units had a low level of ongoing resting discharge averaging 0.08 ± 0.02 i.p.s.. Injection of 5-HT excited five of the six units, which responded with biphasic increase in discharge similar to that described above for in vivo studies (see fig. 6.8). For a standard dose of 10 μg the fast response had a mean latency to onset of 0.5 ± 0.4 seconds and a mean duration of 2.7 ± 0.5 seconds. The peak

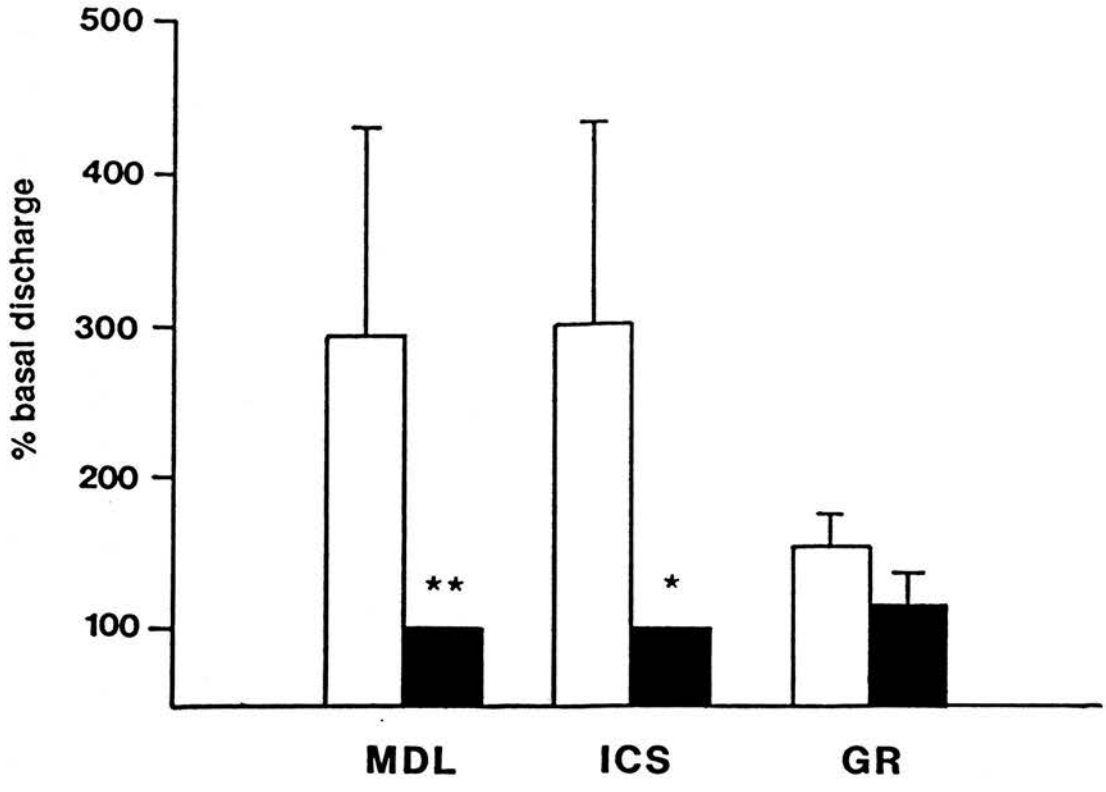


Fig. 6.7 Effects of MDL 72222 (100 μgkg^{-1}, i.a., n = 6), GR38032F (100 μgkg^{-1}, i.a., n = 4) and ICS 205-930 (100 μgkg^{-1}, i.a., n = 4) on 5-HT-induced fast excitation in chemosensitive units from both normal and arthritic rats. Each bar shows the mean peak response as a percentage of basal discharge to a standard effective dose of 5-HT (5 - 100 μg, i.a.) before (open bars) and after (filled bars) injection of antagonist. Error bars represent s.e. mean. Significantly different values are shown as * when $p < 0.05$, and ** when $p < 0.01$ (Wilcoxon).

discharge over a 15 s time period averaged 0.5 ± 0.1 i.p.s.. Repeated injections of 5-HT induced a rapid tachyphylaxis of this response and dose-dependency could not be examined. The delayed excitation had a mean latency to onset of 44 ± 15 seconds and a mean duration of 526 ± 120 seconds. The peak discharge over a 15 s time period averaged 1.8 ± 0.7 i.p.s.. This response could not be shown to be dose-dependent. A summary of the units excited by 5-HT is shown in table 6.3.

6.2.2.2 Excitation of chemosensitive units

In this series of experiments six recordings, consisting of between one and three different action potentials, were obtained from units for which no mechanosensitive receptive fields could be found. These units had a mean resting discharge of 0.9 ± 0.2 i.p.s.. Injection of 5-HT (0.01 - 100 μg, i.a.) excited all units tested. A biphasic response was evoked in two recordings, the slow response was seen alone in three, and in one recording only the fast response was evoked (see table 6.3.). For a standard dose of 10 μg fast excitation had a mean latency to onset of 1.3 ± 0.3 seconds, and a mean duration of 9 ± 5.5 seconds. The peak discharge, obtained over a 15 s time period, had a mean value of 1.6 ± 0.8 i.p.s.. Delayed excitation had a mean latency to onset of 58 ± 25 seconds, and a mean duration of 306 ± 96 seconds. The peak discharge over a 15 s time period averaged 2 ± 0.4 i.p.s.. Fast and slow responses were prone to tachyphylaxis and dose dependency could not be studied.

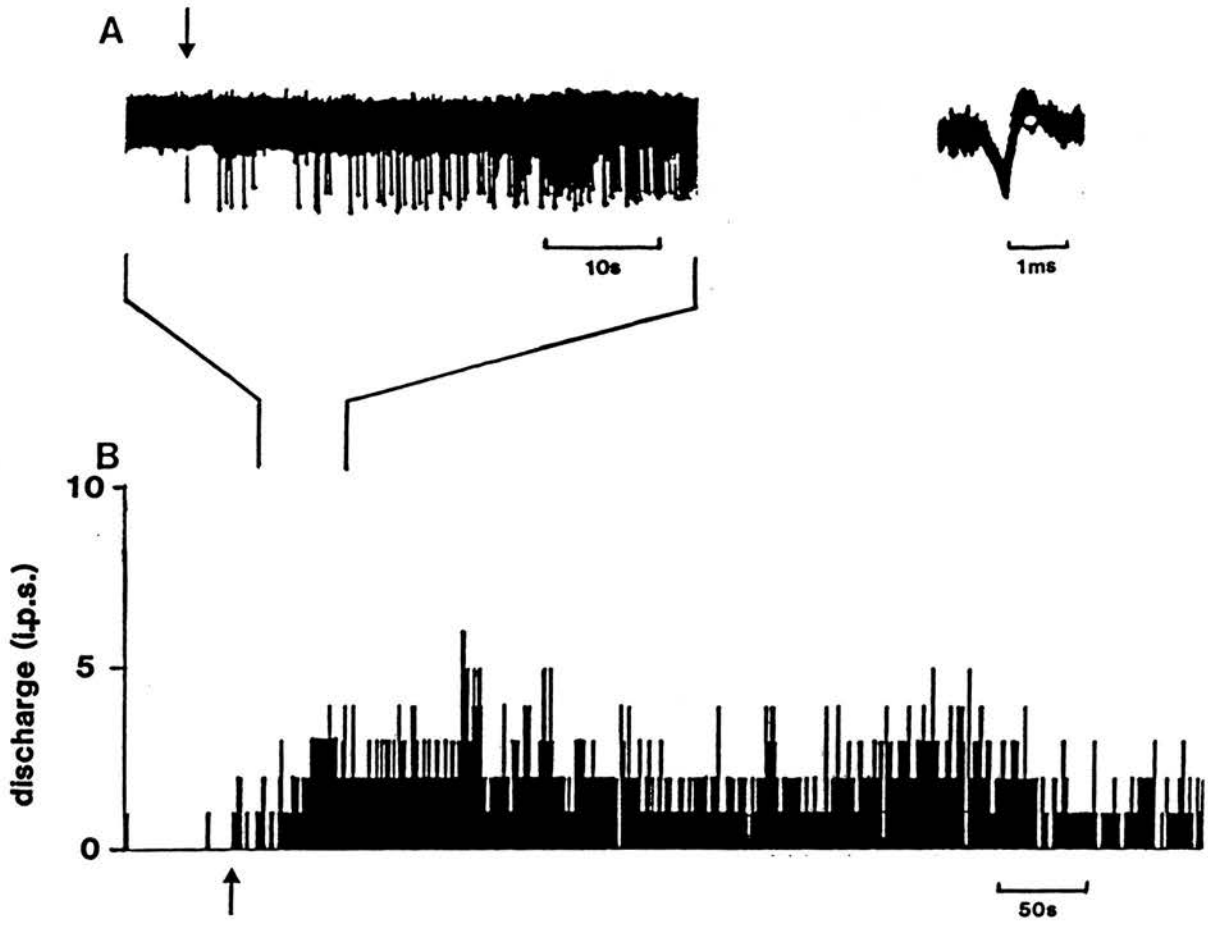


Fig. 6.8 Effects of 10 μg 5-HT on the activity of a mechanonociceptor from a normal joint in vitro. (A) (i) Neurogram showing the discharge pattern of fast and slow excitation evoked by the injection of 5-HT at the arrow. (ii) Shows 30 superimposed triggered oscilloscope sweeps of the single unit action potential which was counted. (B) Computer-generated plot of the discharge evoked by the same injection (at arrow) shown in the neurogram. The time period corresponding to the neurogram is indicated above the graph.

Table 6.3 Summary of the number of mechanonociceptors and chemosensitive units excited by 5-HT *in vitro*.

unit type	no. of units	no. of units displaying each type of response	
		rapid	delayed
mechanonociceptor	6	5 (83%)	5 (83%)
chemosensitive	6	3 (50%)	5 (83%)

The percentage of units responding with each type of response is shown in brackets

6.2.2.3 Effects of 5-HT receptor antagonists on 5-HT-evoked excitation

Mechanonociceptors

In two mechanoreceptors with a high level of ongoing activity evoked by 5-HT, injection of the the 5-HT2 receptor antagonist ketanserin (100 μgkg^{-1} equivalent dose for whole rat, i.a.), caused a marked reduction this activity. This effect had a latency to onset of 60 and 30 seconds and lasted for over 8 minutes in each case. Ongoing discharge was reduced by 97% and 100% for the two units. In one of these units injection of 5-HT evoked a high level of ongoing discharge that was abolished by injection of the 5-HT3 receptor antagonist ICS 205-930 (100 $\mu g/kg$ equivalent dose, i.a.). This effect had a latency to onset of 15 seconds and lasted for over 12 minutes.

The 5-HT3 receptor antagonists could not be studied for their ability to antagonize the fast response evoked by 5-HT because it was prone to tachyphylaxis. In three units the slow response was found to be markedly reduced or abolished by the 5-HT2 receptor antagonist ketanserin (100 $\mu gkg-1$ equivalent dose, i.a.), but in two of these units it was unaffected by the 5-HT3 receptor antagonist ICS 205-930 (100 μgkg^{-1} equivalent).

Chemosensitive units

In one recording with high levels of ongoing activity evoked by 5-HT, injection of the the 5-HT2 receptor antagonist ketanserin (100 μgkg^{-1} equivalent, i.a.), caused a marked reduction in ongoing activity. This

effect had a latency to onset of 45 seconds and lasted for over 8 minutes. Ongoing discharge was reduced by 65%. In the same recording a previous injection of 5-HT evoked a high level of ongoing discharge that was reduced by injection of the 5-HT3 receptor antagonist ICS 205-930 (100 μgkg^{-1} equivalent, i.a.). This effect had a latency to onset of 15 seconds and lasted for over 12 minutes.

The 5-HT antagonists could not be studied for their ability to antagonize fast or slow responses evoked by 5-HT because of their susceptibility to tachyphylaxis.

6.3 DISCUSSION

6.3.1 Excitatory and sensitizing effects

This investigation has shown that exogenous 5-HT can both excite and increase the responsiveness of nociceptive sensory receptors located in the ankle joint capsular tissues of normal and arthritic joints. Mechanonociceptors were affected by 5-HT in normal and arthritic joints, the effect of the amine being greater in the latter. These units displayed a low level of spontaneous activity in inflamed joints, in contrast to the general lack of activity in normal joints. Background discharge in non-mechanosensitive units was present in normal rats, but was much more common in arthritics. These units were activated by 5-HT, and responded with two components - transient excitation followed by a delayed, long lasting increase in activity. The finding that these responses could also be evoked in vitro suggests that they are not

mediated via the release of systemic blood-borne mediators or through spinal reflex mechanisms.

6.3.1.1 Mechanonociceptor sensitization

Single close-arterial bolus injections of 5-HT increased the responses of mechanonociceptors to a standard mechanical stimulus for as long as six minutes. This duration of action is similar to that reported for 5-HT-induced sensitization to the excitatory effects of bradykinin on high-threshold mechanoreceptors in muscle (Mense, 1981) as well as for the sensitizing action of 5-HT on slowly adapting type II cutaneous mechanoreceptors (Fjallbrant & Iggo, 1961). The 5-HT_3 receptor antagonists MDL 72222, ICS 205-930 or GR38032F prevented this action, but did not otherwise affect the response to mechanical stimuli. Ketanserin was without effect. These results suggest that the sensitization demonstrated may involve the action of 5-HT at a 5-HT_3 receptor associated with the terminals of articular mechanonociceptors. The latency (10 -15 s) and the duration of action is consistent with an action involving second messengers in the sensory nerve terminal. Activation of the 5-HT_3 receptor has yet to be linked with the production of a particular second messenger, although 5-HT_1 like or 5-HT_2 receptor activation has been shown to be associated with production of the second messengers IP3 or cAMP in a variety of systems (see Peroutka, 1988). Alternatively the delayed nature of these effects may be related to a 5-HT_3 receptor mediated stimulation of the local production of other sensitizing substances such as the prostanoids (see Section VII).

6.3.1.2 Fast excitation

Brief excitation of mechanonociceptors and chemosensitive units occurred within 10 seconds of the injection of 5-HT both in normal and arthritic joints. This action was blocked or reduced by the 5-HT_3 receptor antagonists MDL 72222, ICS 205-930 or GR38032F.

Fast depolarizations evoked by 5-HT have been reported in several isolated neuronal preparations. In rabbit superior cervical ganglion (SCG) (Wallis & North, 1978), rabbit nodose ganglion (Higashi & Nishi, 1982) and guinea-pig coeliac ganglion (Wallis & Dun, 1988) 5-HT produces a rapid depolarization. These fast depolarizations show marked tachyphylaxis and are sensitive to MDL 72222 or ICS 205-930 (Azami et al., 1985; Round & Wallis, 1985; Round & Wallis, 1986; Wallis & Dun, 1988). 5-HT evoked depolarizations of the rabbit or rat isolated vagus nerve have also been shown to be sensitive to 5-HT_3 antagonists (Donatsch et al., 1984; Richardson et al., 1985)

6.3.1.3 Delayed Excitation

The most consistent response produced by 5-HT was a dose-dependent delayed long lasting increase in discharge that was seen in the majority of the chemosensitive units examined, as well as in mechanonociceptors recorded both in normal and arthritic joints. The 5-HT_3 receptor antagonists had no effect on the slow response, whereas the 5-HT_2 receptor antagonist ketanserin reduced or abolished it. The delayed nature of this effect suggests that 5-HT may be acting indirectly to evoke sustained afferent activity. Delayed excitation was consistent and

dose-dependent in vivo, outlasting the hypotensive effect of 5-HT, thus reducing the liklihood that these effects were produced secondary to blood flow changes. In addition there is evidence that blood flow in the hind-paw of the rat is unaffected by locally administered 5-HT (Owen, 1977). Alternative mechanisms for indirect effects may involve the release of other algogenic substances such as bradykinin (see Section V) or the prostanoids (see Section VII) from surrounding tissues by 5-HT.

Evidence for a direct effect of 5-HT on sensory neurones is supplied by studies on isolated neuronal preparations where a slow response produced by 5-HT has been reported. In C-type neurones from rabbit nodose ganglion a long-lasting depolarization was occasionally seen in response to 5-HT (Higashi & Nishi, 1982). In guinea-pig coeliac ganglion neurones delayed depolarization induced by 5-HT develops slowly and lasts for seconds or minutes (Kiraly et al., 1983; Dun et al., 1984). This response in coeliac ganglion cells is unaffected by MDL 72222 but is partially blocked by methysergide indicating that a 5-HT_3 receptor is not involved (Wallis & Dun, 1988). In rabbit nodose ganglion cells, 5-HT-induced inhibition of of a Ca^{2+}-dependent K^+ conductance is resistant to ICS 205-930 but is antagonized by methysergide (Christian et al., 1989). This effect has previously been reported to be produced by forskolin (Weinreich & Wonderlin, 1987), suggesting that inhibition of the Ca^{2+}-dependent K^+ conductance is mediated via cAMP. This finding, however, does not support a role for 5-HT_2 receptors since the second messenger commonly associated with this receptor type is IP3 (see Peroutka, 1988).

6.3.2 Involvement of 5-HT in nociceptor sensitization during inflammation

The ability of 5-HT to sensitize high threshold C- and A-delta articular mechanonociceptors suggests that endogenous 5-HT could play a role in the increased responsiveness of these receptors in inflamed joints. The results indicate that a 5-HT_3 receptor may be involved. However, in arthritic rats administration of antagonists selective for 5-HT_3 or 5-HT_2 receptors did not significantly reduce mechanonociceptor sensitivity as they should have done if endogenous 5-HT acting at such receptors was the major factor contributing to their heightened responsiveness. Low levels of ongoing activity in mechanonociceptor units observed in arthritic rats were, on the other hand, reduced markedly by the addition of antagonists for 5-HT^3 receptors. This effect lasted only about 5 minutes, a period which is much shorter than the blockade produced by these antagonists of 5-HT responses which lasted for at least 30 min. Similar results were obtained for chemosensitive units following administration of either 5-HT_3 or 5-HT_2 receptor antagonists. These observations suggest that endogenous 5-HT may contribute to ongoing activity seen in inflamed joints, but is by no means the only contributing factor.

For mechanonociceptors from arthritic joints, where mechanical sensitivity is enhanced, responsiveness to 5-HT was much greater than those from normal joints. The minimal effective dose for increased responsiveness to mechanical stimuli was a fifth of that for normal joints and responses lasted over four times as long. In arthritic rats mechanonociceptor units were also excited by doses of 5-HT a hundred

fold smaller than that required in normal joints. These findings provide clear evidence that sensitivity to 5-HT is increased for receptors in inflamed joints, and indicate that the acute release of endogenous 5-HT could further boost sensitivity.

Finally, a role for 5-HT in the development of acute inflammatory pain has been suggested by Eschalier et al. (1989) who have shown that the administration of ICS 205-930 inhibits and reverses carrageenan induced hyperalgesia in rats. It is possible that 5-HT, released from platelets (Garatinni & Valzelli, 1965; Franzen & Eysell, 1969; Page, 1988), mast cells (Johnson & Erdos, 1973) or nerve fibres (Dun et al., 1980; Verhofsted, 1981; Neel & Parsons, 1986) is responsible for development of sensitization during the acute inflammatory response and becomes less important for chronic sensitization. However, further acute release of 5-HT may act to increase levels of sensitization over short periods of time during chronic inflammation.

6.4 CONCLUSIONS

The 5-HT_3 receptor mediated sensitization and rapid excitation of articular mechanonociceptors seen in the present study is consistent with the observation that pain produced by application of 5-HT to a blister base is mediated through a 5-HT^3 receptor (Donatsch et al., 1984; Richardson et al., 1985).

A role for 5-HT_2 receptors in 5-HT-induced pain has not previously been described, and further studies into the origins of the delayed excitation would be of great interest, particularly in view of its

marked and consistent nature in these experiments. The fact that both 5-HT_2 and 5-HT_3 receptor antagonists reduced ongoing activity in arthritic joints suggests that both types of receptor are involved in activity evoked by 5-HT during chronic inflammation. However, the short duration of these effects suggests that 5-HT may not be essential for sustained discharge in this particular model of arthritis.

SECTION VII

EFFECTS OF PROSTANOIDS ON ARTICULAR SENSORY RECEPTORS IN THE RAT ANKLE JOINT

SECTION VII

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7.1 INTRODUCTION

Prostanoids were first proposed to play a role in inflammation by Willis (1978), who discovered large amounts of prostaglandin activity in inflammatory exudates from rat paws injected with carrageenan. Subsequently various different prostanoids were identified in inflamed tissues from both animals and man (see Higgs et al., 1984). A number of studies have reported on the presence of high prostanoid concentrations in the synovium of arthritic joints (Higgs et al, 1974; Robinson et al., 1975; Ferreira, 1979). Studies of this type have been directed mainly towards demonstrating the effects of non-steroidal anti-inflammatory drugs in reducing the production of inflammatory prosatanoids *in vivo* (Higgs et al., 1974; Robinson et al., 1975; Tokunaga et al., 1981).

Prostanoids are thought to contribute significantly to the major signs of inflammation. PGE_1 and PGE_2 cause local vasodilatation and increased vascular permeability when injected into the skin of animals (Horton, 1963; Crunkhorn & Willis, 1971a; Kaley & Weiner, 1971; Peck and Williams, 1978), and man (Crunkhorn & Willis, 1971b; Kuehl, 1971). Prostanoids also contribute to the pain of inflammation, an effect which is generally considered to result from their potentiation of the algesic

actions of other inflammatory mediators such as bradykinin (Ferreira, 1972; Moncada et al., 1975; Ferreira et al., 1978; Ferreira & Nakamura, 1979a; Tyers & Haywood, 1979; Higgs et al., 1984).

Electrophysiological studies in rats with adjuvant-induced polyarthritis have shown that capsular high-threshold C-mechanoreceptors from the ankle joint have an increased sensitivity to mechanical stimuli, and an enhanced level of ongoing discharge (Guilbaud et al., 1985). This enhanced sensitivity and ongoing activity was reduced by lysine-acetylsalicylate (l-AS), suggesting that locally produced cyclooxygenase metabolites may be responsible, at least in part, for the sensitized state (Guilbaud & Iggo, 1985). The aim of the present investigation was to examine the effects of the prostanoids on the properties of articular mechanonociceptors from the ankle joints both of normal rats and those with localized adjuvant-induced arthritis. The effects of l-AS on the sensitivity of nociceptive sensory receptors, and the reversibility of these effects by administration of exogenous prostanoids were studied in rats with localized adjuvant arthritis. The effects of the prostanoids on articular mechanonociceptors from normal joints were also examined, and the prostanoid receptors mediating these effects characterized using a range of naturally occurring prostanoids, and the highly selective IP-receptor agonist cicaprost (Skuballa et al., 1986).

7.2 RESULTS

7.2.1 Normal joints

The effects of PGE_2, PGI_2 and cicaprost were examined *in vivo* on C and A-delta high-threshold mechanoreceptors with receptive fields in the capsular tissue of the tibio tarsal joint. The effects of PGE_2 and cicaprost on responses of these sensory receptors to bradykinin were also examined. Experiments were carried out *in vitro* using the rat isolated hindlimb preparation to examine the effects of PGE_2, cicaprost, PGD_2 and $PGF_{2\alpha}$ both on high-threshold mechanoreceptors and on 'chemosensitive' units for which no mechanoreceptive fields could be found.

7.2.1.1 Electrophysiology *in vivo*

In seventeen experiments, the effects of the prostanoids were examined on the responsiveness of nineteen high-threshold mechanoreceptors to mechanical stimuli. A further three mechanosensitive units were examined for only the excitatory effects of the compounds used. Mechanonociceptor units had afferent fibre conduction velocities in the range 0.4 - 11.5 ms^{-1}. The excitatory and sensitizing effects of the prostanoids on articular mechanonociceptors are summarized in tables 7.1 - 7.8.

Mechanonociceptor excitation

The excitatory effects of PGE_2 were examined on thirteen

mechanonociceptors. These units had either a low level of ongoing resting discharge (mean: 0.6 ± 0.2 i.p.s, n=7), or were silent before drug injection. Injection of PGE_2 (0.3 - 3 μg, i.a.) excited only 23% of the units tested. Table 7.1 shows a summary of the excitatory responses evoked by PGE_2. Excitation had a latency to onset ranging from 0.2 - 4 minutes, and was short lived with a duration of 0.2 - 5.8 minutes. The median minimal effective dose was 0.3 μg, and only those units which were already spontaneously active were excited by PGE_2. Dose-dependency of was not evident in any of the responsive units. The mean peak unit discharge, measured over 60 s, was 1.5 ± 0.6 i.p.s.. Pooled data for maximum responses to PGE_2 are shown in figure 7.1.

The effects of PGI_2 were examined on five mechanonociceptors. These units had either a low level of ongoing resting activity (mean: 0.3 ± 0.2 i.p.s, n=4) or were silent. PGI_2 (0.01 - 0.1 μg, i.a.) excited 80% of units. Figure 7.2 illustrates the pattern of excitation evoked by PGI_2 on a single articular mechanonociceptor. A summary of excitatory responses is shown in table 7.1. There was a latency ranging from 0.5 - 4 minutes before excitation, and the response had a duration ranging from 1 - 24 minutes. The minimal effective dose was 0.1 μg for all four units. Three of the responsive units had a resting discharge before exposure to PGI_2, while the fourth was silent. Dose-dependency of the response was seen in the two units which were examined in this way. The mean peak unit discharge, measured over 60 s, was 0.6 ± 0.3 i.p.s.. Pooled data for maximum responses to PGI_2 are shown in figure 7.1.

Twelve mechanonociceptor units were examined for the excitatory effects of cicaprost. These units had either a low resting discharge (mean: 0.4 ± 0.1 i.p.s., n=7) or were silent. Injection of cicaprost

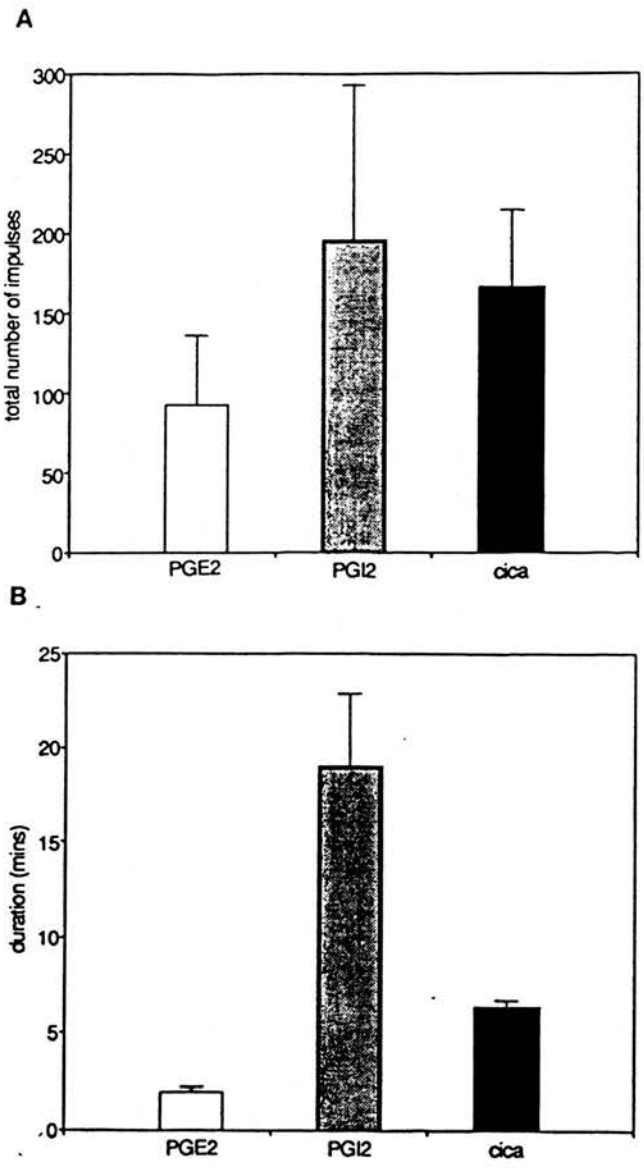


Fig. 7.1 Pooled maximal responses for mechanoreceptor excitation evoked by PGE_2, PGI_2 or cicaprost. (A) Bar graph showing the mean (+ s.e.m.) maximal integrated response (delta Σx) for units excited by injections of PGE_2 (median dose: 3 μg, i.a., n=3), PGI_2 (median dose: 0.1 μg, i.a., n=4) and cicaprost (median dose: 0.5 μg, i.a., n=10). (B) Mean durations of the same responses shown above in A. PGE_2 excited only three of thirteen units (23%), PGI_2 four of five units (80%) and cicaprost eleven of twelve units (92%). The durations of the effects caused by PGI_2 and cicaprost are considerably greater than those for PGE_2, particularly in relation to PGI_2.

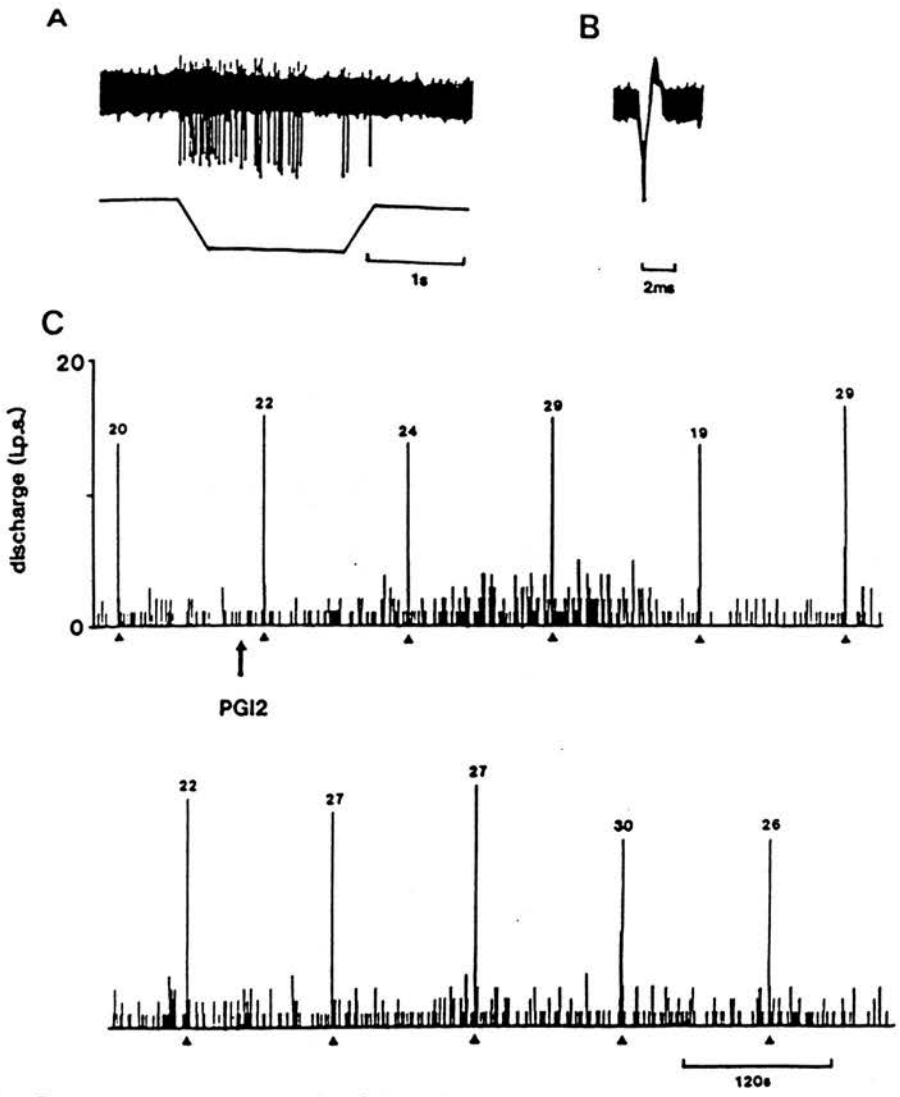


Fig. 7.2 Computer-generated plot illustrating the excitatory and sensitizing effects of PGI_2 on a single articular mechanoreceptor with an afferent fibre conduction velocity of 0.44 ms^{-1}. (A) Neurogram showing the response of this receptor to a single mechanical indentation stimulus. (B) thirty superimposed triggered oscilloscope sweeps of the single action potential which was counted (C) Mechanical stimuli were applied for 2 s once every 2 mins (arrowheads) before and after the injection of PGI_2 (0.1 μg, i.a.) at the arrow. Each bar represents a one second time interval, with the lower trace being a continuation of that in the upper panel. The number of action potentials evoked per mechanical stimulus is shown above each response. A long lasting excitation and sensitization to mechanical stimuli was produced following the injection of PGI_2.

Table 7.1 Summary of the relative numbers of articular mechanonociceptors excited by PGE_2, PGI_2 or cicaprost.

prostanoid	min. effective dose (μg)	no. of units	no. of units excited	response latency (mins)	response duration (mins)
PGE_2	0.3 - 3	13	3 (23%)	3.3 ± 0.7	1.9 ± 0.2
PGI_2	0.1	5	4 (80%)	2.7 ± 0.9	15.4 ± 4.8
cicaprost	0.05 - 0.1	12	11 (92%)	1.3 ± 0.35	5.2 ± 1.3

The percetage of mechanonociceptors excited is given in brackets

(0.01 - 5 μg, i.a.) excited 92% of units. Table 7.1 shows a summary of the excitatory responses evoked by cicaprost. There was a latency to onset ranging from 0.1 - 5.7 minutes, and the response had a duration ranging from 0.3 - 23 minutes. The median minimal effective dose was 0.1 μg. Seven of the responsive units had a resting discharge before exposure to cicaprost, while the remaining four were silent. Dose-dependency of the response was seen in six of seven units tested in this way. The mean peak discharge, measured over 60 s, was 1.7 ± 0.4 i.p.s.. A summary of maximum unit responses is shown in figure 7.1.

Comparison of units tested with more than one prostanoid agonist revealed that two units excited by PGI_2 were also excited by cicaprost, and two units not excited by PGE_2 were excited by cicaprost.

Mechanonociceptor responsiveness

The effects of PGE_2 (0.03 - 3 μg, i.a.) were examined on thirteen mechanonociceptors. Their responsiveness to mechanical stimuli, applied once every two minutes, was increased significantly (sensitization) in 46% of units. The time course for the onset, time to peak and duration of the response varied between units. A summary of the sensitizing effects of PGE_2 on mechanical responsiveness is shown in table 7.2. The minimal effective dose had a median value of 3 μg. The peak unit response, calculated from values taken at different time points following the injectionn of PGE_2, had a mean value of 174% of the pre-injection control. Figure 7.3 illustrates pooled data for the maximal increase in responsiveness caused by PGE_2.

In five units (38%) the highest dose used (3 μg), caused a marked

Table 7.2 Summary of the relative numbers of articular mechanonociceptors sensitized to mechanical stimuli by PGE_2, PGI_2 or cicaprost.

prostanoid	min. effective dose (μg)	no. of units	no. of units sensitized	response latency (mins)	response duration (stimuli)
PGE_2	0.03 - 3	13	6 (46%)	0.25 - 4	1 - 3
PGI_2	0.1	5	4 (80%)	0.25 - 4	1 - 17
cicaprost	0.05 - 1	8	7 (88%)	0.25 - 2	1 - 12

The percentage of mechanonociceptors excited is given in brackets
Response durations are given as the range over which responsiveness to mechanical stimuli (applied once every 2 mins) was increased.

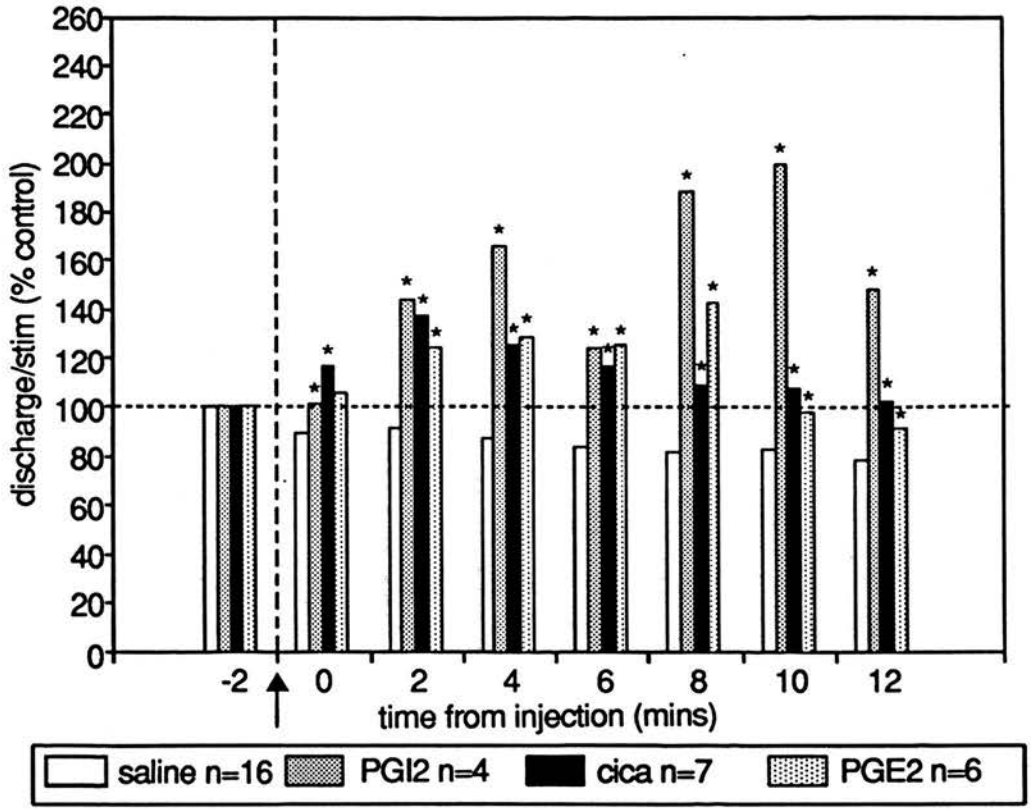


Fig. 7.3 Poooled maximal responses for mechanical sensitization evoked by PGE_2, PGI_2 or cicaprost. The bars represent mean responses of mechanoreceptors to mechanical stimuli, applied once evey two minutes, expressed as a percentage of the pre-injection control response (=100%). PGE_2 (median dose: 0.3 μg, i.a.,) sensitized six of thirteen units (43%), PGI_2 (median dose: 0.1 μg, i.a.,) four of five units (80%), and cicaprost (median dose: 0.1 μg, i.a.) seven of eight units (88%). The time at which the injection was made is indicated by the arrow and the vertical dashed line. Values significantly greater than those obtained following injection of saline vehicle are shown as * when $p< 0.05$ (Wilcoxon). Responses to PGI_2 were particularly marked and of long duration.

reduction in responsiveness to mechanical stimuli. In two of these units lower doses of PGE_2 (0.03 -0.3 μg, i.a.) had caused a sensitization to mechanical stimuli. Reduced responsiveness had a latency to onset of 0.25 minutes, and lasted for over 10 minutes in all five units. The mean minimal unit response, calculated from values taken at different time points following the injection, was 63% of pre-injection control. The pooled maximal reduction in responsiveness obtained following injection of PGE_2 is illustrated in figure 7.4. The three units described above that were sensitized to mechanical stimuli were also excited by PGE_2.

The effects of PGI_2 (0.01 - 1 μg, i.a.) were examined on five mechanonociceptors. Their responsiveness to mechanical stimuli, applied once every two minutes, responsiveness was increased significantly in 80% of these units. An example of the sensitizing effect of PGI_2 is illustrated in figure 7.2. Mechanical sensitization had a duration much longer than that caused by PGE_2. A summary of the sensitizing effects of PGE_2 is shown in table 7.2. The minimal effective dose was 0.1 μg, thirty times less than the minimal effective dose for PGI_2. Dose-dependency of the response was tested in two units, both of which gave larger responses with increasing dose. The mean peak response, calculated from values taken at differet times after injection, was 228% of the pre-injection control, nearly twice that caused by PGE_2 at higher doses. Figure 7.3 illustrates the pooled maximal responses obtained following injection of PGI_2. The one unit that was not sensitized to mechanical stimuli by PGI_2 was also not excited.

The effects of cicaprost (0.05 - 5 μg, i.a.) were examined on eight mechanonociceptors. Their responsiveness to mechanical stimuli, applied once every two minutes, was increased significantly for seven (88%).

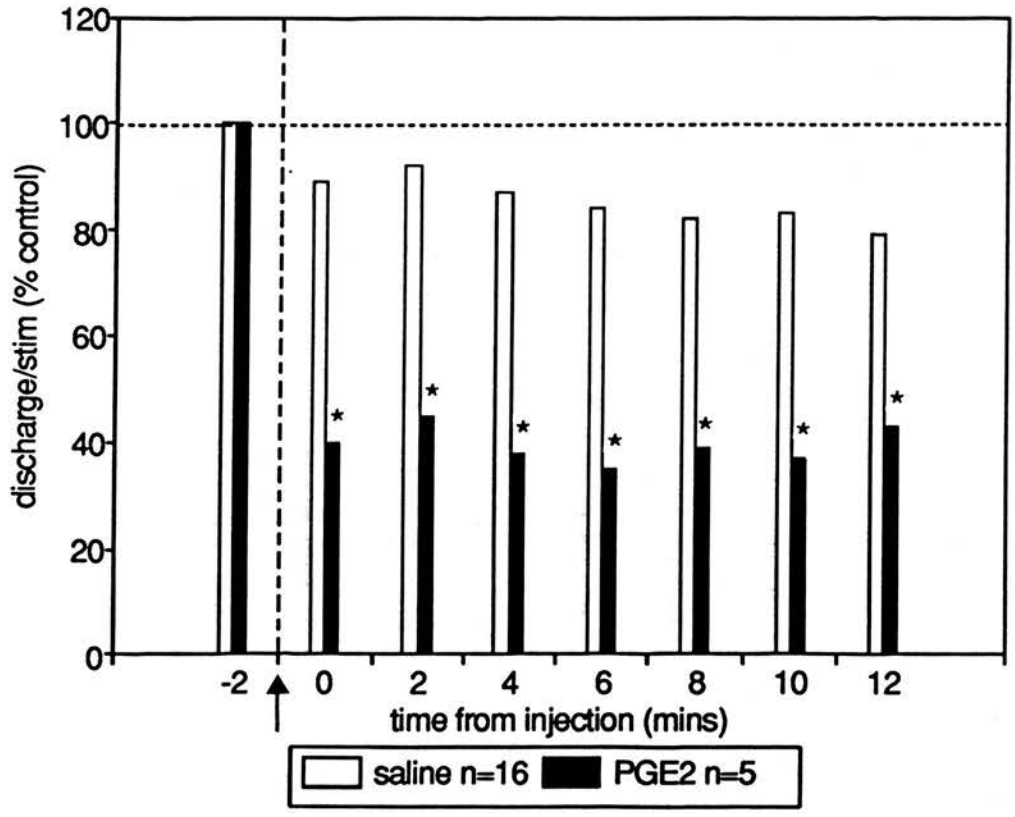


Fig. 7.4 Pooled data showing the maximal reduction in mechanical responsiveness evoked by PGE_2. Bars represent mean responses of the five mechanoreceptors to mechanical stimuli, applied once every two minutes, expressed as a percentage of the pre-injection control response. Injection of PGE_2 (3 μg, i.a.) evoked a reduction in the mechanical responsiveness of these five units. The arrow and vertical dashed line show the time point at which the injection was made. Values significantly lower than those obtained following injection of saline vehicle are shown as * when $p < 0.05$ (Wilcoxon).

Increased responsiveness was seen after a variable delay and had a duration intermediate to sensitization caused by PGE_2 and PGI_2. A summary of the mechanical sensitization caused by PGI_2 is shown in table 7.2. The minimal effective dose was of the same order of magnitude as for PGI_2, and had a median value of 0.1 μg. Dose-dependency was tested in three units, all of which gave larger responses with increasing dose. The mean peak response, caculated from values at different time points following injection, was 164% of the pre-injection control, less than that caused by PGI_2. Figure 7.3 illustrates the pooled maximal responses obtained following injection of cicaprost. One unit sensitized to mechanical stimuli was not excited by cicaprost.

Comparison of units tested with more than one prostanoid agonist revealed that two units sensitized to mechanical stimuli by PGI_2 were also sensitized by cicaprost, and from four units tested with PGE_2 and cicaprost two units not sensitized by PGE_2 were sensitized by cicaprost.

Combined effects of prostanoids and bradykinin: mechanonociceptor excitation

The effects of combined injections of threshold or subthreshold excitatory doses of bradykinin (0.1 - 10 μg, i.a.) and PGE_2 (0.03 - 3 μg, i.a.) were studied in nine mechanonociceptors. These units had either a low level of ongoing resting discharge (mean: 0.5 ± 0.1 i.p.s., n=6), or were silent before the injection of any drugs. The excitatory responses evoked by the prostanoids, bradykinin and combined injections are summarized in tables 7.3 - 7.5. Injection of PGE_2 evoked an irregular discharge in only one (11%) of the nine units. The mean peak

Table 7.3 Summary of the excitatory effects of combined injections of PGE_2 and bradykinin on articular mechanonociceptors.

drug	dose range (μg)	no. of units	no. of units excited	response latency (mins)	response duration (mins)
PGE_2	0.03 - 3	9	1 (11%)	4	3.8
BK	0.1 - 10	9	3 (33%)	2.3 ± 0.6	4 ± 0.5
PGE_2/BK	0.03-3/0.1-10	9	5 (56%)	2.2 ± 1.0	7.2 ± 2.0

The percentage of mechanonociceptors excited is given in brackets

Table 7.4 Summary of the excitatory effects of combined injections of PGI_2 and bradykinin on articular mechanonociceptors.

drug	dose range (μg)	no. of units	no. of units excited	response latency (mins)	response duration (mins)
PGI_2	0.01 - 0.1	5	4 (80%)	2.1 ± 0.8	19 ± 4
BK	10	5	4 (80%)	2.2 ± 1.3	5.3 ± 1.2
PGI_2/BK	0.01-0.1/10	5	5 (100%)	1.8 ± 1.0	10.7 ± 1.6

The percentage of mechanonociceptors excited is given in brackets

Table 7.5 Summary of the excitatory effects of combined injections of cicaprost and bradykinin on articular mechanonociceptors.

drug	dose range (μg)	no. of units	no. of units excited	response latency (mins)	response duration (mins)
cicaprost	0.05 - 0.1	7	1 (14%)	2	8
BK	1 - 10	7	1 (14%)	0.05	0.5
cica/BK	0.05-0.1/1 - 10	7	6 (86%)	1 ± 0.4	7.2 ± 0.6

The percentage of mechanonociceptors excited is given in brackets

discharge, determined over a 60 s recording period, was 0.9 ± 0.2 i.p.s.. Injection of bradykinin excited 33% of the units. Excitation had a latency to onset ranging from 1.2 - 3.2 minutes, and a duration ranging from 3.3 - 5 minutes. The mean peak discharge, measured over a one minute period, was 0.9 ± 0.2 i.p.s.. Combined injections of bradykinin and PGE_2 evoked excitation greater than that produced by either drug alone in 55% of units. These effects were greater than additive in all cases. Responses had a latency to onset ranging from 0.67 - 4.8 minutes, and a duration ranging from 1.7 - 10.8 minutes. The mean peak discharge, measured over 60 s, was 0.8 ± 0.2 i.p.s. Figure 7.5 shows a summary of the responses evoked by each drug alone and by combined injections.

The effects of combined injections of threshold or subthreshold doses of bradykinin (10 μg, i.a.) and PGI_2 (0.01 - 0.1 μg, i.a.) were examined in five mechanonociceptors. These units had either a low level of ongoing resting discharge (0.3 ± 0.2 i.p.s., n=4), or were silent before the injection of any drugs. Injection of PGI_2 excited 80% of units. Excitation had a latency to onset ranging from 0.5 - 4 minutes and a duration ranging from 7 - 24 minutes. The mean peak discharge, determined over 60 s, was 0.6 ± 0.2 i.p.s.. Injection of bradykinin excited 80% of the units. Excitation had a latency to onset ranging from 0.1 - 5.3 minutes, and a duration ranging from 2 - 8 minutes. The mean peak discharge, measured over 60 s, was 0.7 ± 0.3 i.p.s.. Combined injections of bradykinin and PGI_2 evoked excitation greater than that produced by either drug alone in only 40% of units. These effects were greater than additive. In the remaining three units PGI_2 evoked a high level of activity when given alone. The response to combined injections

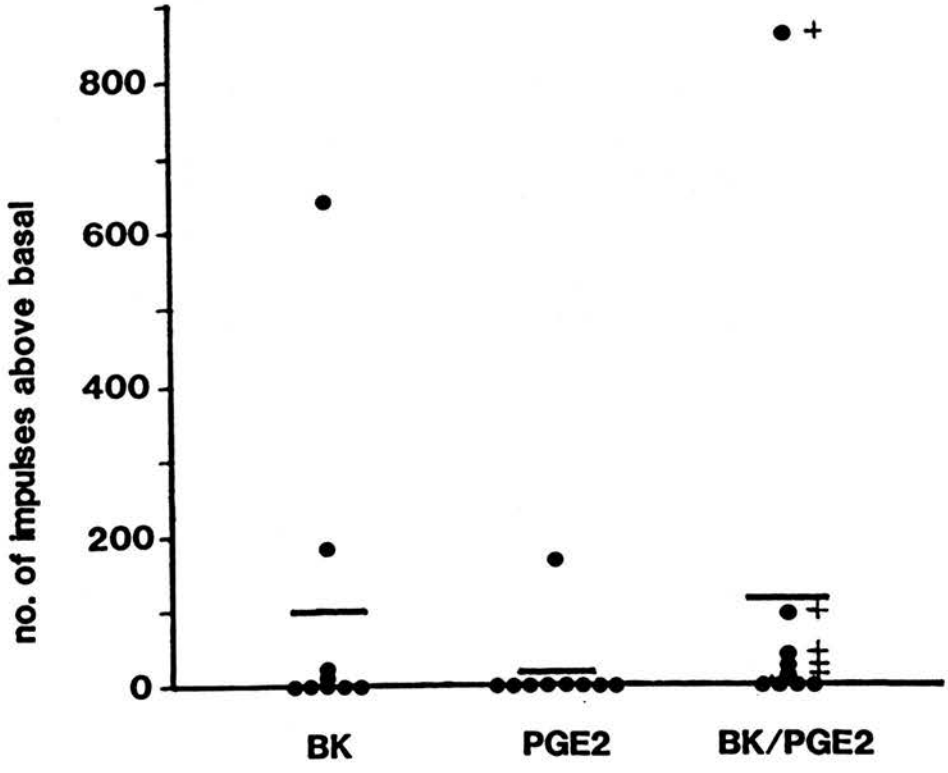


Fig. 7.5 Summary of the excitatory effects of combined injections of threshold and sub-threshold doses of bradykinin and PGE_2 on articular mechanonociceptors. Each point on the scatter diagram represents the integrated response (delta Σx) of a single unit to the injection of either bradykinin, PGE_2, or combined injections of the same doses of each drug. The horizontal bars represent the mean evoked response, and the crosses indicate units for which combined injections evoked a response greater than that caused by either drug alone. Although these effects were greater than additive in all five units the mean response for combined injections was not significantly greater than the sum of the mean responses to each drug alone ($p < 0.05$, Wilcoxon).

had a latency to onset ranging from 0.1 - 6 minutes, and lasted for 4 - 16.6 minutes. The mean peak discharge, measured over 60 s was 0.9 ± 0.4 i.p.s. Figure 7.6 summarizes the responses evoked by each drug alone and by combined injections.

The effects of combined injections of threshold or subthreshold doses of bradykinin (1 - 10 μg, i.a.) and cicaprost (0.05 - 0.1 μg, i.a.) were studied in seven mechanosensitive units. These units had either a low level of ongoing resting discharge (0.5 ± 0.3 i.p.s., n=4), or were silent before the injection of any drugs. Injection of cicaprost excited one (14%) of the seven units. Excitation had a latency to onset of 120 seconds and lasted for 8 minutes. The peak discharge, determined over a one minute recording period, was 0.3 i.p.s.. Injection of bradykinin also excited one (14%) unit. Excitation had a latency to onset of 3 seconds, a duration of 30 seconds, and evoked a peak discharge, measured over a one minute period, of 0.1 i.p.s.. Combination injections of bradykinin and cicaprost evoked excitation greater than that produced by either drug alone in six (86%) of the seven units. These effects were greater than additive in all cases. The response had a latency to onset ranging from 0.25 - 2.7 minutes, and lasted for 5 - 9.2 minutes. The mean peak unit discharge, measured over 60 s, was 2.6 ± 1.2 i.p.s.. Figure 7.7 summarizes the responses evoked by each drug alone and by combined injections. An example of the effect of cicaprost or bradykinin alone or in combination is illustrated in figure 7.8.

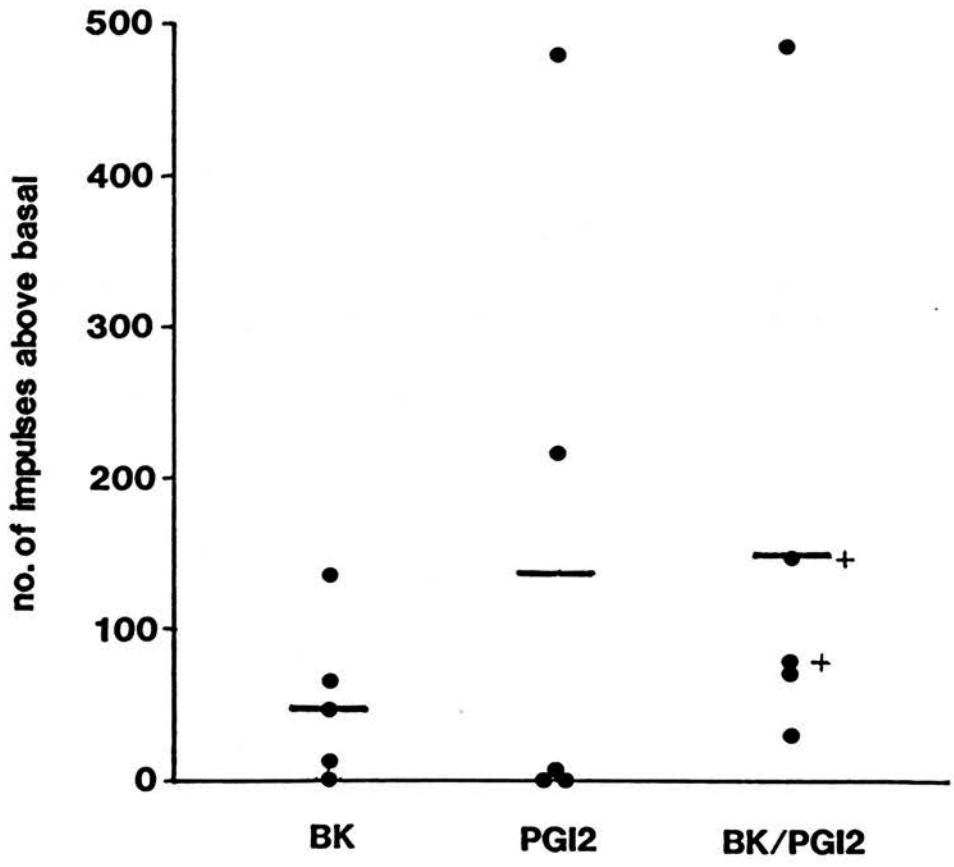


Fig. 7.6 Summary of the excitatory effects of combined injections of threshold and sub-threshold doses of bradykinin and PGI_2 on articular mechanonociceptors. Each point on the scatter diagram represents the integrated response (delta Σx) of a single unit to the injection of either bradykinin, PGI_2, or combined injections of the same doses of each drug. The horizontal bars represent the mean evoked response, and the crosses indicate units for which combined injections evoked responses greater that that caused by either drug alone. Although these effects were greater than additive in all five units the mean response for combined injections was not significantly greater than the sum of the mean responses to each drug alone ($p < 0.05$, Wilcoxon).

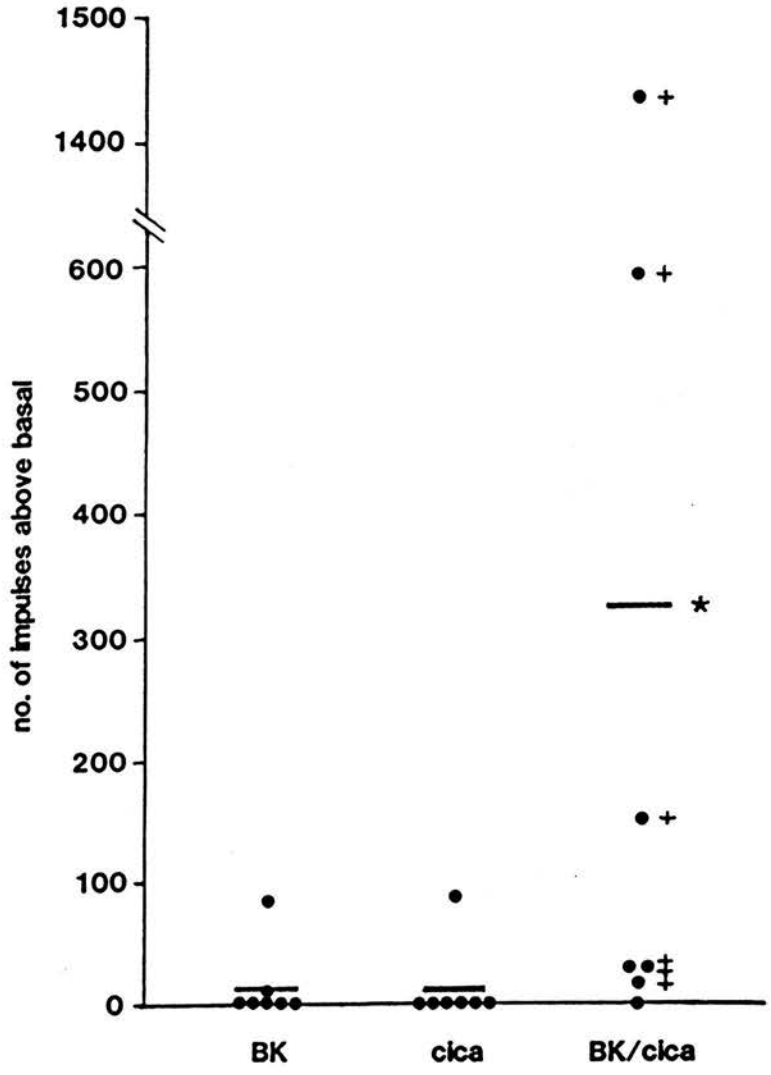
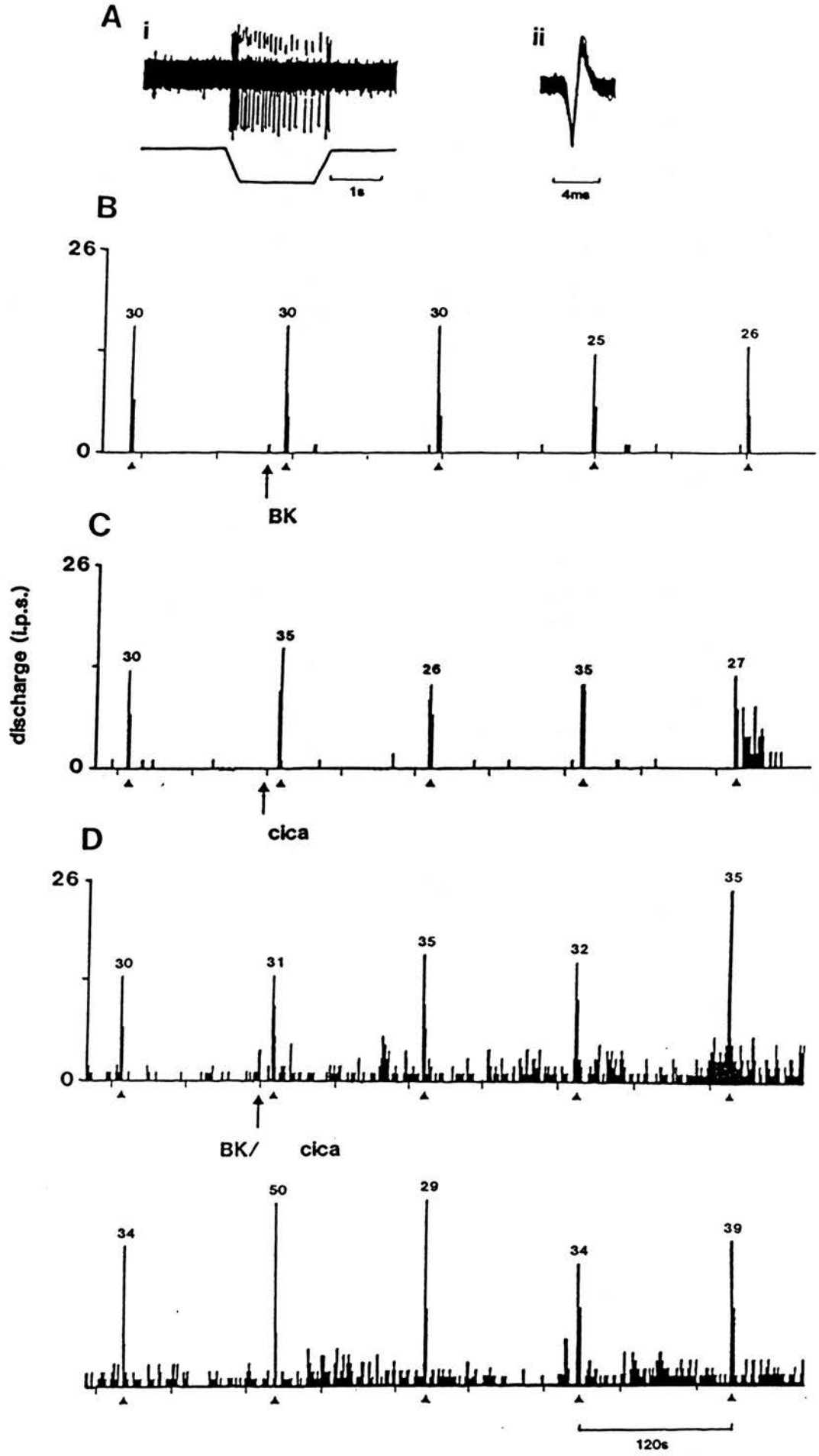


Fig. 7.7 Summary of the excitatory effects of combination injections of threshold and sub-threshold doses of bradykinin and cicaprost on articular mechanoreceptors. Each point on the scatter diagram represents the integrated response (delta Σx) of a single unit to the injection of either bradykinin, cicaprost, or combined injections of the same doses of each drug. The horizontal bars represent the mean evoked response, and the crosses indicate units for which combined injections evoked a response greater that that caused by either drug alone. These effects were considerably greater than additive in all six units, the mean response evoked by combined injections being significantly (* $p < 0.05$, Wilcoxon) greater than the sum of the mean responses evoked by each drug alone.

Fig. 7.8 Computer-generated plot illustrating the excitatory and sensitizing effects of injections of cicaprost or bradykinin alone and a combined injection on a single articular mechanonociceptor with an afferent fibre conduction velocity of 0.44 ms^{-1}. (A) (i) Neurogram showing the response of this unit to a mechanical indentation stimulus (waveform shown below neurogram) (ii) Thirty superimposed triggered oscilloscope sweeps of the action single potential that was counted. The bars in each of the graphs represent a 1 s time interval, with consecutively labelled graphs being a continuation of the one above. Mechanical stimuli of 2 s duration were applied once every two minutes (arrowheads) before and after the injection of (B) bradykinin (10 μg, i.a.), (C) cicaprost (1 μg, i.a.) and (D) the combination of the two doses. Injections are indicated by the arrows below each trace. The number of impulses evoked by mechanical stimuli is shown above each response. The graphs show that bradykinin alone had little effect, cicaprost alone evoked a transient increase in mechanical responsiveness and a delayed (6 mins) excitation, and a combined injection evoked long lasting mechanical sensitization and mechanonociceptor excitation.


A
i
ii
1s
4ms
B
26
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30
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25
26
BK
C
discharge (i.p.s.)
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0
30
35
26
35
27
cica
D
26
0
30
31
35
32
35
BK/
cica
34
50
29
34
39
120s

Combined effects of prostanoids and bradykinin: mechanical responsiveness

The sensitizing effects of the prostanoids, bradykinin and combined injections are summarized in tables 7.6 - 7.8. The effects of combined injections of threshold or subthreshold doses of bradykinin (0.5 - 10 μg, i.a.) and PGE_2 (1 - 3 μg, i.a.) on responsiveness to mechanical stimuli were studied in nine units. Injection of PGE_2 caused increased responsiveness in only one (11%) of the nine units. The peak response obtained following the injection was 117% of the pre-injection control. In five units (56%) PGE_2 caused a reduction in responsiveness to mechanical stimuli. This response had a delay to onset of 15 seconds, and lasted for 2 - 8 stimuli. Injection of bradykinin caused increased responsiveness in 56% of units. The mean response at the peak of the effect, calculated from values taken at different times following injection, was 126% of pre-injection control. Combined injections of bradykinin and PGE_2 caused greater sensitization than that produced by either drug alone in (89%) of units. These effects were greater than additive in all cases. The mean response at the peak of the effect was 164% of pre-injection control. Figure 7.9 shows pooled data for the sensitization to mechanical stimuli caused by each drug alone and in combination.

The effects of combined injections of subthreshold or threshold doses of bradykinin (10 μg, i.a.) and PGI_2 (0.01 - 0.1 μg, i.a.) on responsiveness to mechanical stimuli were studied in five units. Injection of PGI_2 caused increased responsiveness in 80% of these units. The mean peak response, calculated from values taken at different times

Table 7.6 Summary of the sensitizing effects of combined injections of PGE_2 and bradykinin on the mechanical responsiveness of articular mechanonociceptors.

drug	dose range (μg)	no. of units	no. of units sensitized	response latency (mins)	response duration (stimuli)
PGE_2	1 - 3	9	1 (11%)	0.25	3
BK	0.5 - 10	9	5 (56%)	2 - 4	3 - 4
PGE_2/BK	1-3/0.5-10	9	8 (89%)	0.25 - 6	1 - 6

The percentage of mechanonociceptors sensitized is given in brackets
Response durations are given as the range over which responsiveness to mechanical stimuli was increased.

Table 7.7 Summary of the sensitizing effects of combined injections of PGI_2 and bradykinin on the mechanical responsiveness of articular mechanonociceptors.

drug	dose range (μg)	no. of units	no. of units sensitized	response latency (mins)	response duration (stimuli)
PGI_2	0.01 - 0.1	5	4 (80%)	0.25 - 2	1 - 15
BK	10	5	4 (80%)	0.25	1
PGI_2/BK	0.01-1/10	5	5 (100%)	0.25 - 6	2 - 7

The percentage of mechanonociceptors sensitized is given in brackets
Response durations are given as the range over which responsiveness to mechanical stimuli was increased.

Table 7.8 Summary of the sensitizing effects of combined injections of cicaprost and bradykinin on the mechanical responsiveness of articular mechanonociceptors.

drug	dose range (μg)	no. of units	no. of units sensitized	response latency (mins)	response duration (stimuli)
cicaprost	0.1 - 1	5	3 (60%)	0.25 - 8	2 - 3
BK	1 - 10	5	4 (80%)	0.25 - 2	1 - 4
cica/BK	0.1-1/1-10	5	4 (80%)	2 - 4	4 - 8

The percentage of mechanonociceptors sensitized is given in brackets
Response durations are given as the range over which responsiveness to mechanical stimuli was increased.

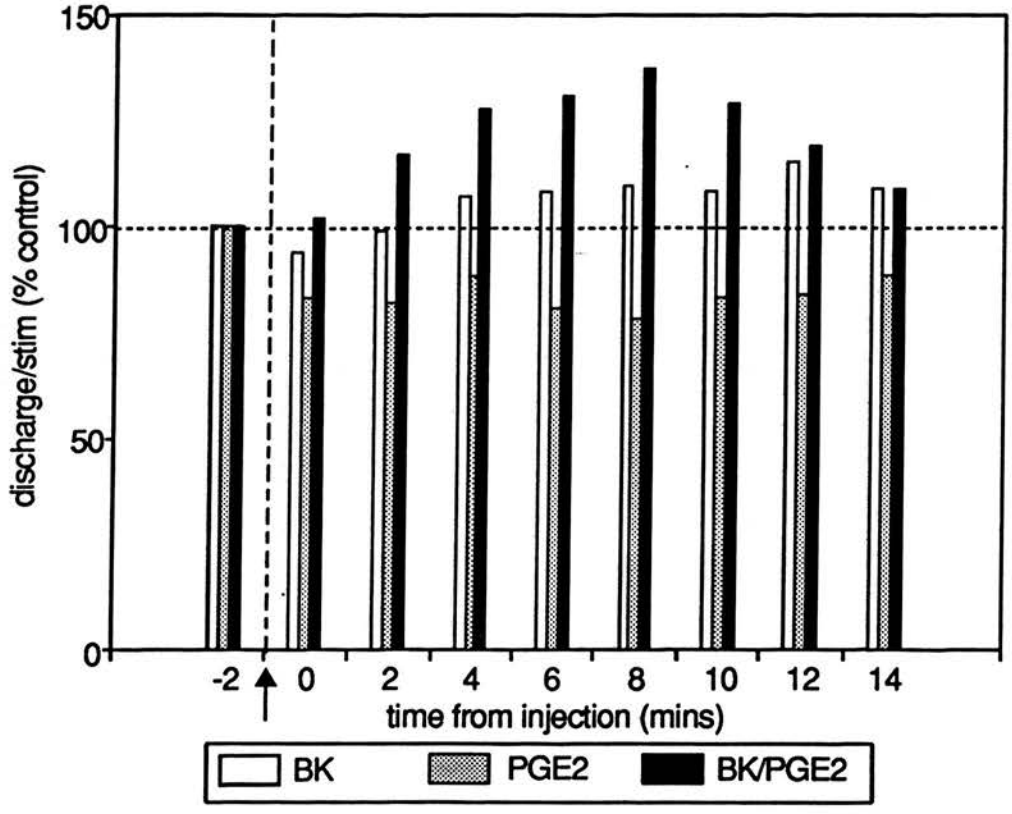


Fig. 7.9 Pooled data showing changes in mechanical responsiveness evoked by threshold and subthreshold doses of bradykinin or PGE_2 alone and combined injections on eight articular mechanonociceptors. Bars represent the mean responses of the eight units to mechanical stimuli of 2 s duration applied once every 2 mins. The time at which the injections were made is shown by the arrow and the vertical dashed line. Although injections of PGE_2 alone caused a depression of mechanical responsiveness, this same dose markedly potentiated the sensitization evoked by bradykinin. Although effects were greater than additive for individual units, mean values at each time point for sensitization evoked by combined injections were not significantly greater than the sum of the sensitization evoked by each drug alone ($p < 0.05$, Wilcoxon).

following injection, was 216% of the pre-injection control. Injection of bradykinin caused increased responsiveness in 80% of units. The mean peak response was 120% of pre-injection control. Combination injections of bradykinin and PGI_2 caused greater sensitization than that produced by either drug alone in four (80%) of the five units. These effects were greater than additive in all four cases. The mean peak response obtained was 185% of the pre-injection control response. In the remaining unit PGI_2 alone produced a marked and sustained sensitization. Figure 7.10 shows pooled data for the sensitization to mechanical stimuli caused by each drug alone and combination.

The affects of combined injections of subthreshold or threshold doses of bradykinin (1 - 10 μg, i.a.) and cicaprost (0.1 - 1 μg, i.a.) on responsiveness to mechanical stimuli were examined in five units. Injection of cicaprost caused increased responsiveness in 60% of units. The mean peak response obtained following the injection was 147% of the pre-injection control. Injection of bradykinin caused increased responsiveness in 80% of units. The mean peak response was 126% of pre-injection control. Combined injections of bradykinin and cicaprost caused greater sensitization than that produced by either drug alone in four (80%) of the five units. These effects were greater than additive in all cases. The mean peak response obtained folloing combined injections was 158% of control. Figure 7.11 summarizes the sensitization to mechanical stimuli caused by each drug alone and in combination.

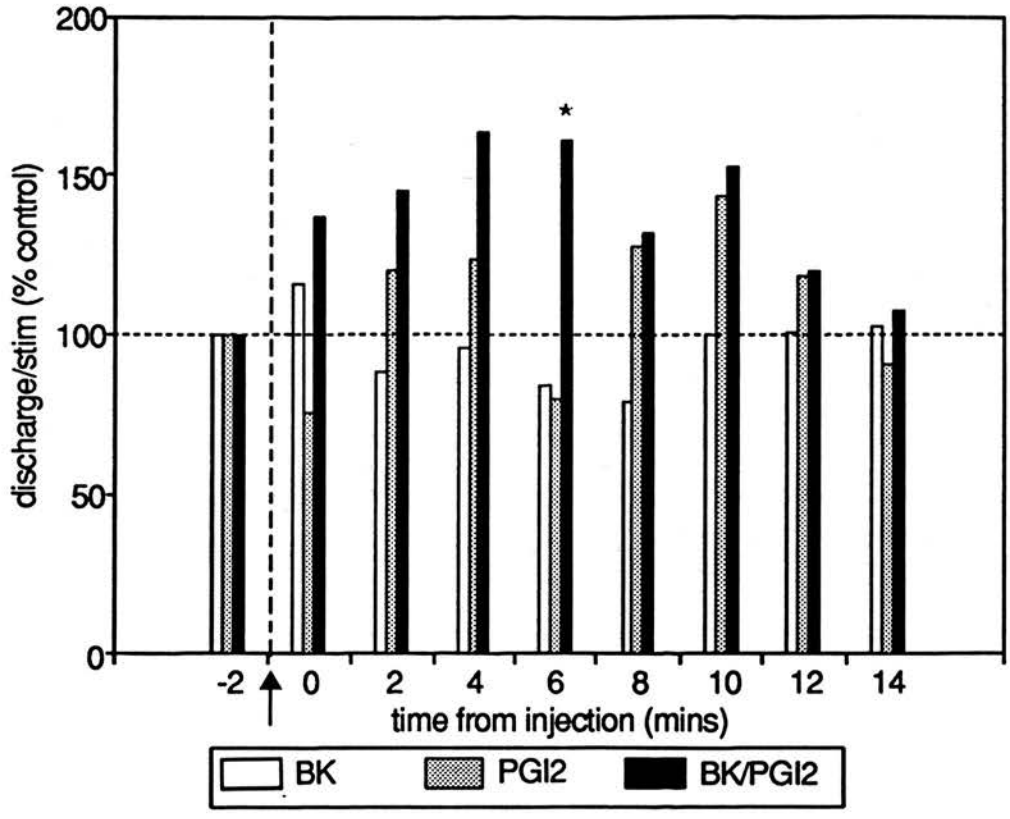


Fig. 7.10 Pooled data showing changes in mechanical responsiveness evoked by injections of threshold and sub-threshold doses of bradykinin or PGI_2 alone and combined injections on five articular mechanonociceptors. Bars represent the mean responses of the five units to mechanical stimuli of 2 s duration applied once every 2 mins. The time of at which injections were made is shown by the arrow and the vertical dashed line. Sensitization to mechanical stimuli evoked by bradykinin was potentiated by PGI_2, although PGI_2 alone caused a sensitization. Mean values significantly different from additive are shown as * when $p < 0.05$ (Wilcoxon).

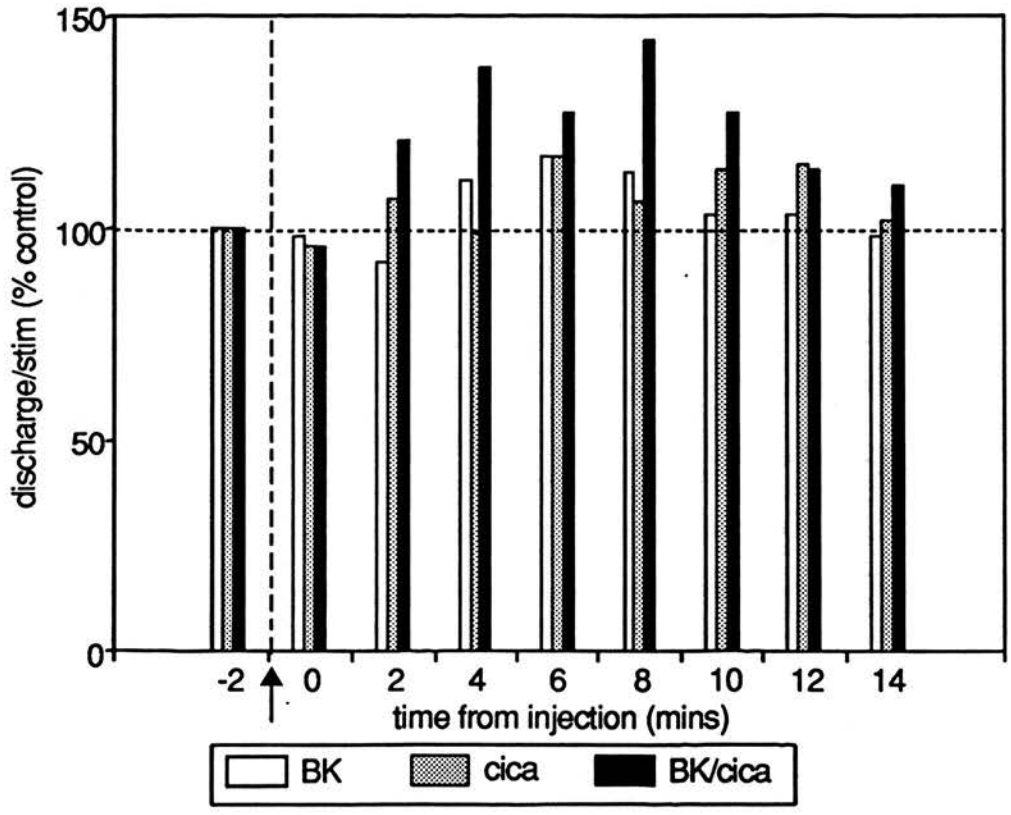


Fig. 7.11 Pooled data showing changes in mechanical responsiveness caused by injections of threshold and sub-threshold doses of bradykinin or cicaprost alone or combined injections on four articular mechanoreceptors. Bars represent the mean responses of four units to mechanical stimuli of 2 s duration applied once every 2 mins. The time at which injections were made is indicated by the arrow and the vertical dashed line. Combined injections of the two agents evoked greater than additive effects on mechanical responsiveness. Although effects were greater than additive for individual units, mean values at each time point for sensitization evoked by combined injections were not significantly greater than the sum of the sensitization evoked by each drug alone ($p < 0.05$, Wilcoxon).

7.2.1.2 Electrophysiology *in vitro*

Using the rat isolated hindlimb preparation the excitatory effects of the prostanoids PGE_2, PGD_2, $PGF_{2\alpha}$ and the PGI_2 analogue cicaprost were examined in thirteen experiments. Mechanical stimuli were not used in these experiments. Both mechanonociceptors and chemosensitive units were studied. In the same experiments the affects of these prostanoid agonists on responses to bradykinin were also investigated. All units examined had afferent fibre conduction velocities in the C fibre range (0.4 - 2 ms^{-1}), or had action potential spike shape characteristics of identified C or fine A-delta afferent units (see Section III).

Mechanonociceptor excitation

The effects of PGE_2, PGD_2 and $PGF_{2\alpha}$ were examined in eight, three and two experiments respectively. A summary of the excitatory effects of the prostanoids on articular mechanonociceptors is shown in table 7.9. From six mechanosensitive units that were excited by capsaicin or 5-HT (see Section IV), injection of PGE_2 (0.01 - 10 μg, i.a.) did not evoke excitation of any of these units. Injections of PGD_2 (0.1 - 10 μg, i.a.) or $PGF_{2\alpha}$ (0.1 - 1 μg, i.a.) failed to excite three mechanoreceptors that were excited by 5-HT or capsaicin (see Section IV).

In eight experiments, cicaprost (0.01 - 1 μg, i.a.) excited six of six units that were excited by capsaicin or 5-HT. The response had a latency to onset ranging from 0.2 - 9 minutes, and had a duration of 3.7 - 27 minutes. The median minimal effective dose was 0.1 μg. Two of the responsive units had a resting discharge (mean: 0.6 ± 0.1 i.p.s.) before

Table 7.9 Summary of the relative numbers of articular mechanonociceptors excited by PGE_2, PGI_2 or cicaprost in vitro.

prostanoid	min. effective dose (μg)	no. of units	no. of units excited	response latency (mins)	response duration (mins)
PGE_2	-	8	0 (0%)	-	-
cicaprost	0.01 - 1	6	6 (100%)	2.6 ± 1.2	13.6 ± 3.4
PGD_2	-	3	0 (0%)	-	-
$PGF_{2\alpha}$	-	2	0 (0%)	-	-

The percetage of mechanonociceptors excited is given in brackets
- indicates that no data was obtained for these parameters

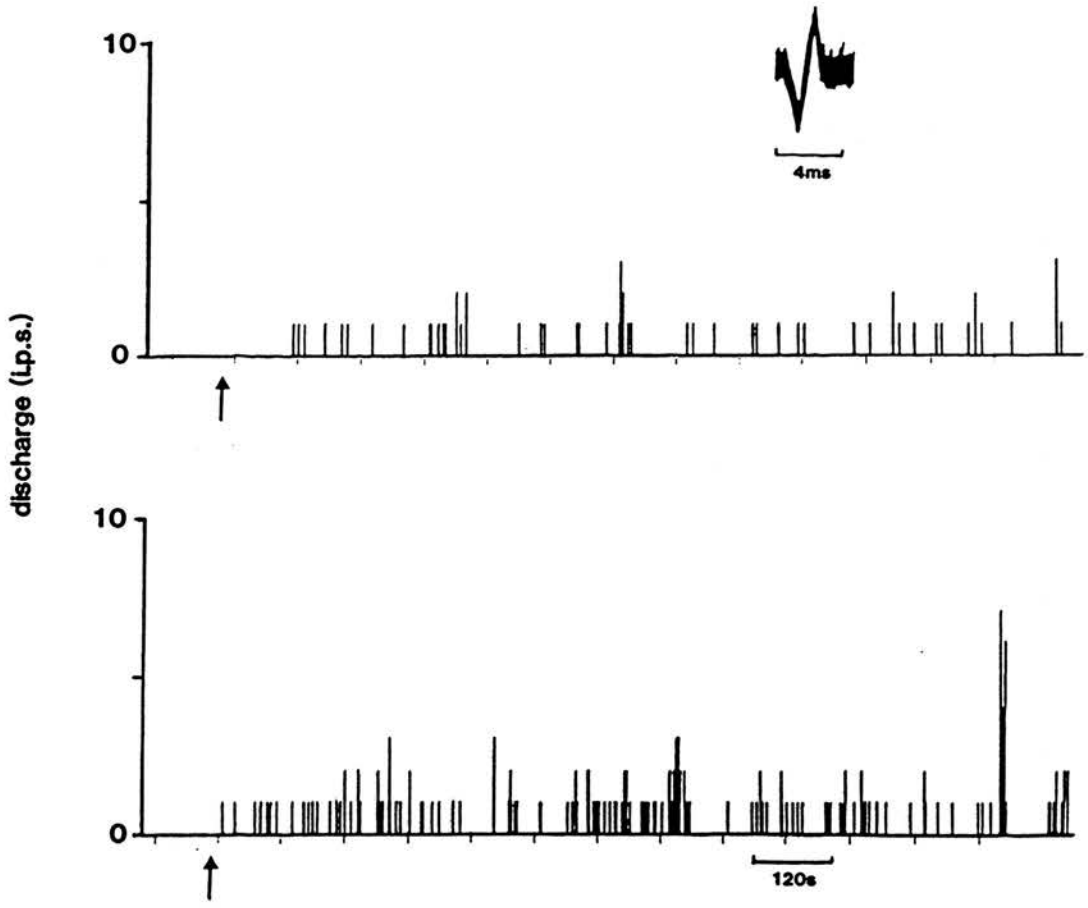


Fig. 7.12 Computer-generated plot illustrating dose-dependent excitation evoked by cicaprost on an articular mechanonociceptor in vitro. The upper trace shows the response to i.a. injection of 0.1 μg cicaprost (at arrow) and the lower trace the response of the same unit to a subsequent i.a. injection of 1.0 μg cicaprost (at arrow). A period of 15 mins elapsed between the injections. Each bar represents a 1 s time interval. The inset trace shows thirty superimposed oscilloscope sweeps triggered by the single action potential that was counted.

exposure to cicaprost. Dose-dependency of the response was seen in the two units which were examined for this relationship. An example of the dose dependent excitation caused by cicaprost is illustrated in figure 7.12. The mean discharge at the peak of the response, measured over 60 s, was 1.3 ± 0.5 i.p.s., and the mean maximum number of evoked action potentials above basal discharge (delta Σx) was 240 ± 118 impulses (mean duration: 13.8 ± 7.2 mins).

Excitation of chemosensitive units

The effects of PGE_2, PGD_2 and $PGF_{2\alpha}$ were examined in eight, three and two experiments respectively. A summary of the excitatory effects of the prostanoids on chemosensitive units is shown in table 7.10. From six recordings, consisting of between one and three different unit action potentials, injection of PGE_2 (0.01 - 10 μg, i.a.) caused excitation in all cases (table 7.10). The response had a latency to onset ranging from 0.3 - 4 minutes, and response had a duration of 1.5 - 9 minutes. The median minimal effective dose was 0.1 μg. Responsive units had a mean resting discharge of 0.6 ± 0.2 i.p.s. before exposure to PGE_2. Dose-dependency of the response was seen in three of the six recordings. The mean discharge at the peak of the response, measured over 60 s, was 2.2 ± 0.6 i.p.s.. Pooled data of the maximal responses evoked by PGE_2 is shown in figure 7.13. Injections of PGD_2 (0.1 - 10 μg, i.a.) or $PGF_{2\alpha}$ (0.1 - 1 μg, i.a.) did not evoke an excitation in any of the recordings.

In eight experiments, cicaprost (0.01 - 1 μg, i.a.) caused excitation in six of six recordings in which activity was evoked by capsaicin (table 7.10). The response had a latency to onset ranging from 0.2 - 5

Table 7.10 Summary of the relative numbers of articular chemosensitive receptors excited by PGE_2, PGI_2 or cicaprost *in vitro*.

prostanoid	min. effective dose (μg)	no. of units	no. of units excited	response latency (mins)	response duration (mins)
PGE_2	0.01 - 1	6	6 (100%)	2 ± 0.4	4.3 ± 1.0
cicaprost	0.01 - 1	6	6 (100%)	1.4 ± 0.5	7.6 ± 2.3
PGD_2	-	3	0 (0%)	-	-
$PGF_{2\alpha}$	-	2	0 (0%)	-	-

The percetage of chemosensitive units excited is given in brackets
- indicates that no data was obtained for these parameters

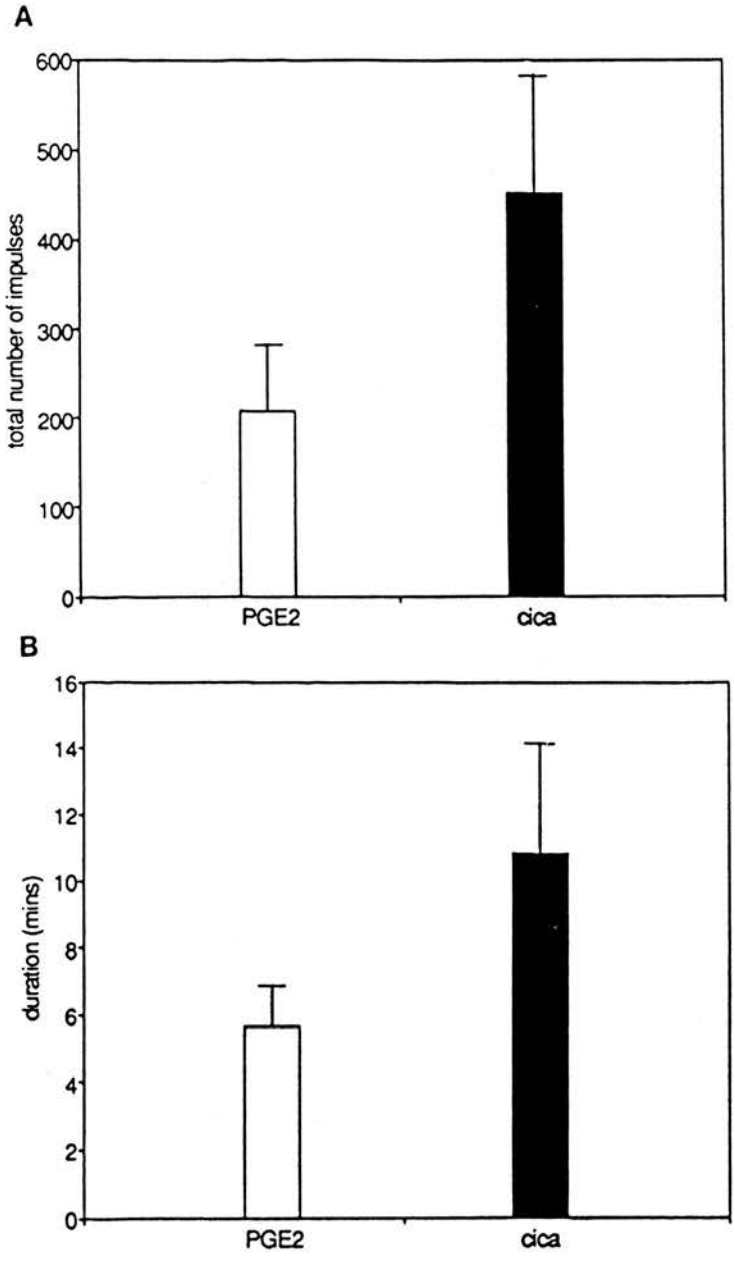


Fig. 7.13 Pooled maximal responses for excitation of chemosensitive units evoked by PGE_2 or cicaprost <u>in vitro</u>. (A) Bar graph showing the mean maximal integrated response (delta Σx) for units excited by injections of PGE_2 (shaded bar) (median dose: 0.1 μg, i.a., n=6), and cicaprost (open bar) (median dose: 0.1 μg, i.a., n=6). (B) Bar graph showing the mean durations of the same responses shown above in A.

minutes), and had a duration of 1 - 27 minutes, approximately twice the duration of the mean PGE_2 response. The median minimal effective dose was 0.1 μg. Responsive units had a mean resting discharge of 0.6 ± 0.2 i.p.s. before exposure to cicaprost. Dose-dependency of the response was seen in two of the six recordings. The mean peak discharge, measured over 60 s, was 3.1 ± 0.6 i.p.s.. Pooled data from maximal responses evoked by cicaprost is shown in figure 7.13.

Combined effects of prostanoids and bradykinin: mechanonociceptors

The effects of PGE_2, PGD_2 and $PGF_{2\alpha}$ on mechanoreceptor responses to bradykinin were examined in eight, three and two experiments respectively. Bradykinin (10 μg, i.a.) excited only one mechanonociceptor from nine. From six tested with PGE_2 (0.01 - 10 μg, i.a.), and three tested with PGD_2 (0.1 - 10 μg, i.a.) or $PGF_{2\alpha}$ (0.1 - 1 μg, i.a.), responses to bradykinin were unaffected in all cases.

From the six mechanoreceptors tested with cicaprost responses to combined injections of threshold or subthreshold doses of bradykinin (1 - 10 μg, i.a.) and cicaprost (0.1 - 1 μg, i.a.) were larger than those evoked by either drug alone in four units. Effects were greater than additive in all cases. An example of the effect of bradykinin or cicaprost alone and combined injection is illustrated in figure 7.14. Respones evoked by combined injections had a mean latency to onset of 2.9 ± 2.6 minutes (range: 0.02 - 16 minutes. The effect had a mean duration of 10.2 ± 3.1 minutes (range: 4.2 - 25 mins), and the mean peak discharge, measured over 60 s, was 2.3 ± 1 i.p.s.. Figure 7.15 summarizes the effects of threshold or subthreshold doses of bradykinin

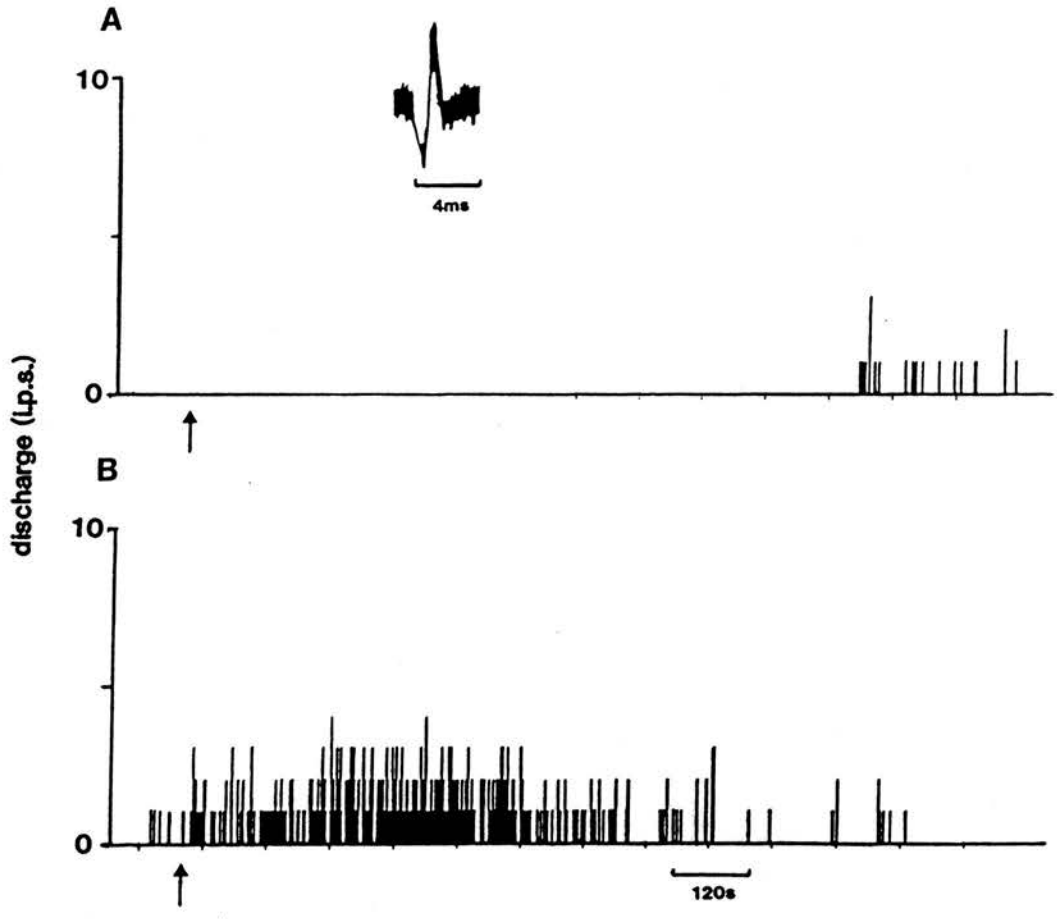


Fig. 7.14 Computer-generated plot illustrating the excitatory effect of cicaprost alone or in combination with bradykinin on an articular mechanonociceptor. Each bar represents a one second time interval (A) Response to i.a. injection of 0.1 μg cicaprost (at arrow). (B) Response to a combined i.a. injection of 0.1 μg cicaprost and a previously innefective dose of 1.0 μg bradykinin (at arrow). The inset trace shows thirty superimposed oscilloscope sweeps triggered by the single action potential that was counted.

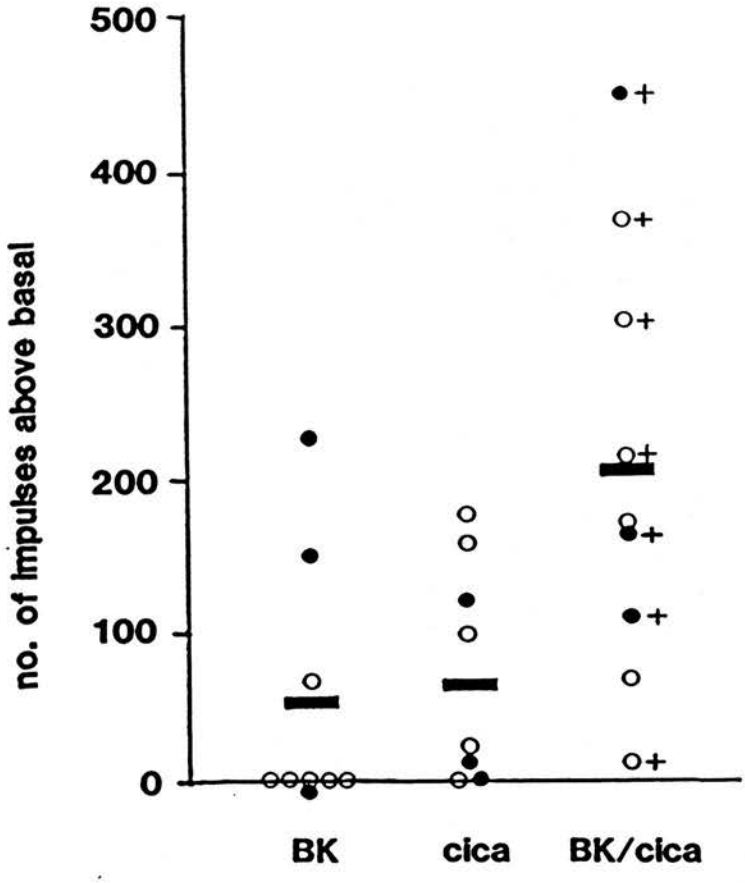


Fig. 7.15 Summary of the excitatory effects of injections of threshold and sub-threshold doses of bradykinin or cicaprost alone and combined injections on articular mechanonociceptors and chemosensitive receptors in vitro. Each point on the scatter diagram represents the integrated response (delta Σx) of a single unit to the injection of either bradykinin, cicaprost, or combined injections of the same doses of each drug. Open circles represent mechanonociceptors, and closed circles represent chemosensitive units. The horizontal bars represent the mean evoked response, and the crosses indicate units for which combined injections evoked a response much greater that that caused by either drug alone. These effects were greater than additive in all seven units.

or cicaprost alone, and combined injections of the two drugs.

Combined effects of prostanoids and bradykinin: chemosensitive units

Injections of PGD_2 (0.1 - 10 μg, i.a.) or $PGF_{2\alpha}$ (0.1 - 1 μg, i.a.) did not cause any change in bradykinin evoked excitation in any of the recordings. From six recordings, combined injections of PGE_2 (0.1 - 10 μg, i.a.) and bradykinin (0.1 - 10 μg, i.a.) caused excitation greater than that produced by either drug alone in three cases. This effect was not any greater than additive. Excitation evoked by combined injections had a mean latency to onset of 0.7 ± 0.3 minutes (range: 0.03 - 2 mins), and the response had a mean duration of 6.6 ± 1.4 minutes (range: 4 - 11.7 mins). The mean peak discharge, measured over 60 s, was 2.2 ± 0.9 i.p.s.. Figure 7.16 shows a summary of responses to threshold or subthreshold doses of bradykinin or PGE_2 alone, and combined injections of the two drugs.

From five recordings, combined injections of cicaprost (0.01 - 0.1 μg, i.a.) and bradykinin (0.1 - 10 μg, i.a.) caused excitation greater than that produced by either drug alone in three cases. This effect was greater than additive in all cases as illustrated in figure 7.15. In the remaining two units cicaprost (0.1 μg, i.a.) evoked a large excitation when given alone. Excitation caused by combined injections had a mean latency to onset of 1.9 ± 1.1 minutes (range: 0.2 - 6 mins), and the response had a mean duration of 9.4 ± 3.9 minutes (range: 1.2 - 24 mins). The mean peak discharge, measured over 60 s, was 2.8 ± 0.5 i.p.s.. Results are summarized in figure 7.15.

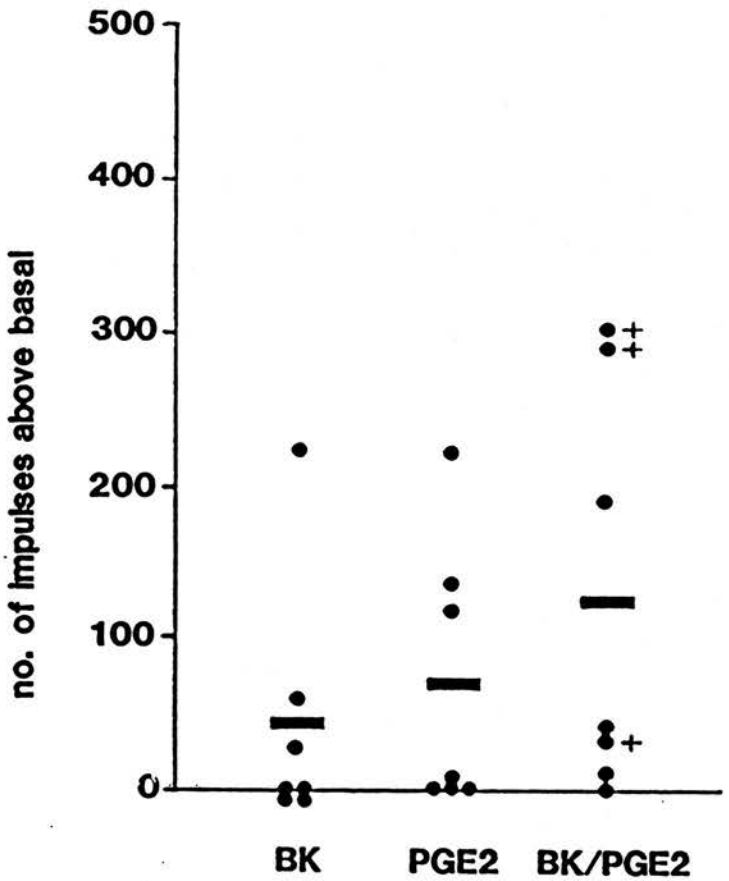


Fig. 7.16 Summary of the excitatory effects of injections of threshold and sub-threshold doses of bradykinin or PGE_2 alone or combined injections on chemosensitive receptors <u>in vitro</u>. Each point on the scatter diagram represents the integrated response (delta Σx) of a single unit to the injection of either bradykinin, PGE_2 or combination injections of the same doses of each drug. The horizontal bars represent the mean evoked response, and the crosses indicate units for which combined injections evoked a response greater that that caused by either drug alone. These effects were only marginally greater than additive.

7.2.2 Arthritic joints

The effects of PGE_2, or the highly selective IP-receptor agonist cicaprost, were examined *in vivo* on C and A-delta articular mechanonociceptor in arthritic joints following the administration of lysine acetylsalicylate (l-AS) to supress endogenous prostanoid production. The effects of l-AS on these receptors were also examined in arthritic joints *in vitro* using the rat isolated hindlimb preparation.

7.2.2.1 Electrophysiology *in vivo*

Studies on the effects of administration of l-AS, PGE_2 or cicaprost were performed in nine experiments, from which sixteen articular mechanonociceptors were identified and studied. Mechanonociceptor units had afferent fibre conduction velocities in the C or fine A-delta range (0.5 - 4.6 i.p.s), and had a mean resting discharge of 2 ± 0.7 i.p.s (n=12).

Effects of l-AS on resting discharge

Injection of l-AS (100 $mgkg^{-1}$, equivalent to 50 $mgkg^{-1}$ acetylsalicylic acid (ASA), i.v.), caused a reduction in resting activity in all twelve units examined. Afferent unit discharge began to decline 2 - 12 minutes after administration, and reached a minimum level after a delay of 4 - 16 minutes (mean: 10 ± 1.5 mins). The mean minimum discharge level attained, measured at different time points following injection, was 36% of pre-injection control. Recovery to higher levels of resting discharge

was not seen for observation periods of up to 40 minutes. Figure 7.17 illustrates a summary of the responses of the twelve units to 1-AS.

Effects of prostanoids on resting discharge following 1-AS

The effects of PGE_2 were examined on ten mechanonociceptors whose resting activity had been reduced by 1-AS. Injection of PGE_2 (0.03 - 30 μg, i.a.), transiently increased afferent discharge to levels approaching those seen before administration of 1-AS in six (60%) of these units. This effect had a mean latency to onset of 2 ± 0.4 minutes, and was generally short lived, with a mean duration of 3.4 ± 0.8 minutes (range: 1 - 11 mins). Dose dependency of the response was seen in all five of the units examined for this relationship, results from which are shown in figure 7.18. The maximum dose used in the majority of units was 3 μg, and pooled data from responses to this dose is shown in figure 7.19. In one unit tested with 30 μg PGE_2, a large (peak discharge: 219% of pre-1-AS control) and sustained (11 mins) increase in resting activity was evoked.

The effects of cicaprost were examined on eight mechanoreceptors whose activity had been reduced by 1-AS. Injection of cicaprost (0.1 - 1 μg, i.a), evoked a large and sustained afferent discharge in seven (88%) of these units. This effect had a relatively short latency to onset (mean : 0.8 ± 0.1 mins), and had a mean duration of 12.5 ± 2.6 minutes (range 3 - 40 mins). Dose dependency of the response was seen in all four units examined for this relationship, results from which are shown in figure 7.20. The maximum dose used in all units was 1 μg, and pooled data of responses to this dose are shown in figure 7.20.

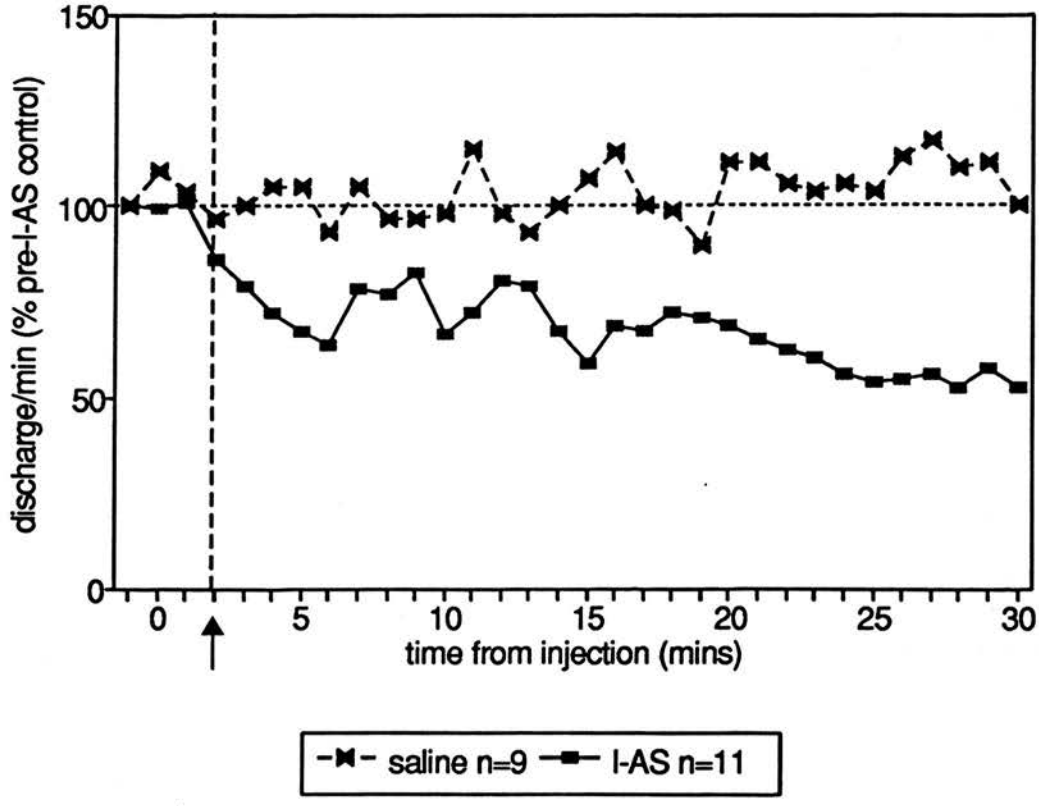


Fig. 7.17 Pooled data showing the depressant effects of l-AS on spontaneous discharge of articular mechanonociceptors in arthritic joints. Each point on the graph represents the mean of the average unit discharge recorded over a 60 s period, and is expressed as a percentage of the pre-injection control (=100%). The arrow and vertical dotted line indicate the time at which injections were made. The effects of i.v. l-AS (50 $mgkg^{-1}$, ASA equivalent) and i.v. saline are shown. Mean values are significantly ($p < 0.05$, Wilcoxon) lower than corresponding values obtained following injection of saline vehicle from 3 mins after l-AS injection.

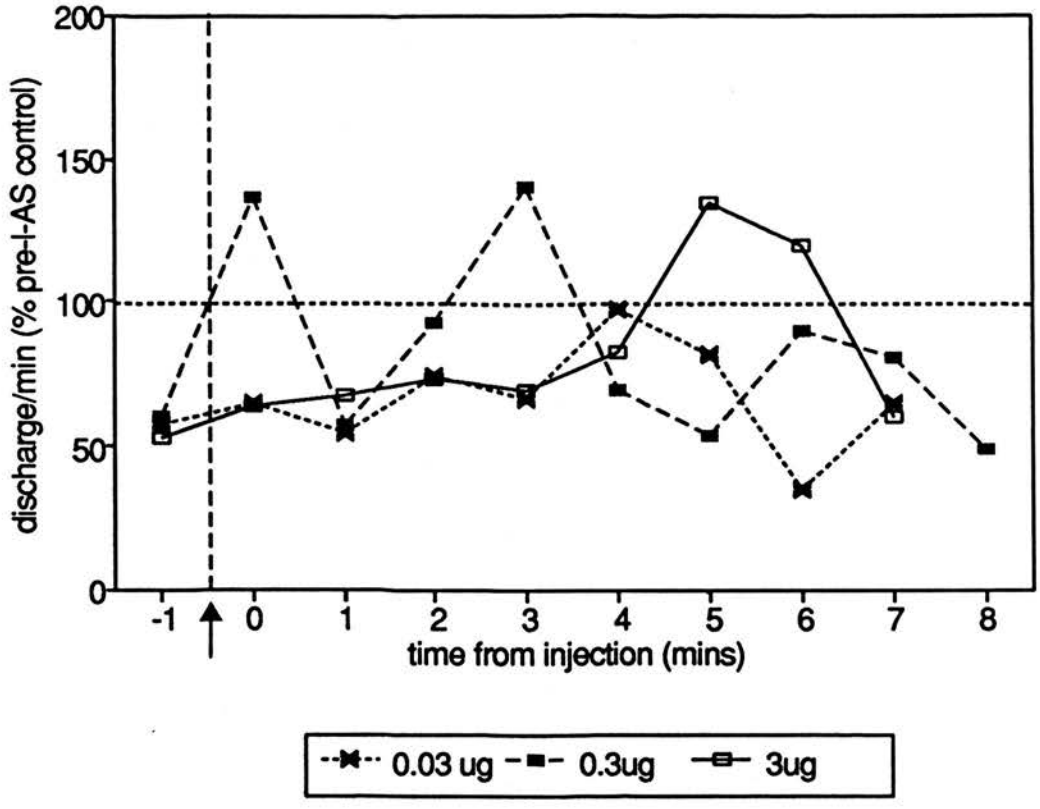


Fig. 7.18 Excitatory effects of increasing doses of PGE_2 on the discharge of articular five mechanonociceptors following treatment with 1-AS. Each point represents the mean of the average unit discharge recorded over a one minute time period. Values are expressed as a percentage of the pre-1-AS control discharge (=100%). The arrow and dashed vertical line indicate the time at which injections of PGE_2 were made. Dose-dependency is seen for doses between 0.03 μg and 0.3 μg, but responses to 3 μg PGE_2 are delayed in onset and on average reduced.

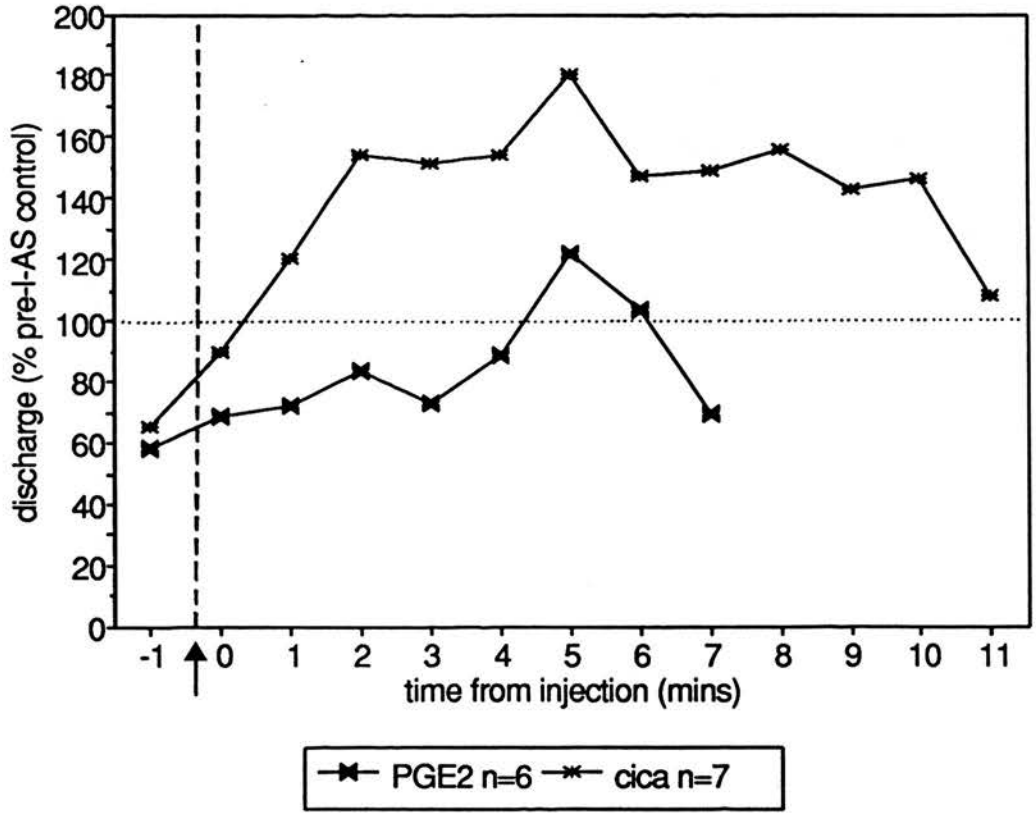


Fig. 7.19 Pooled data showing the maximal excitatory response evoked by PGE_2 or cicaprost following treatment with l-AS. Each point represents the mean discharge recorded over a 60 s period. Values are expressed as a percentage of the pre-l-AS control discharge (=100%). The arrow and dashed vertical line indicate the time at which injections were made. PGE_2 (3 μg, i.a.) caused an increase in afferent discharge in 60% of units, whereas cicaprost (1 μg, i.a.) excited 88%. Only those values for responsive units are shown. The mean maximum response to cicaprost was of greater amplitude and of longer duration than that caused by a higher dose of PGE^2.

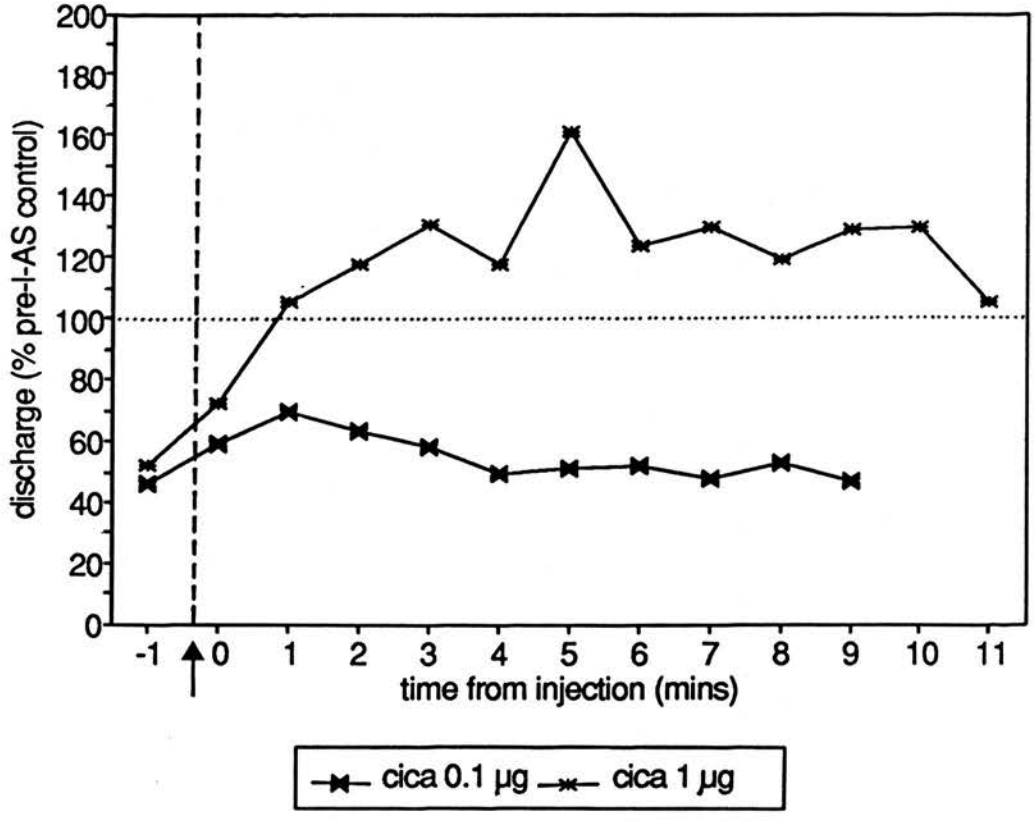


Fig. 7.20 Dose-dependent excitatory effects of cicaprost on the resting discharge of four articular mechanonociceptors following treatment with 1-AS. Each point represents the mean unit discharge recorded over a 60 s period. Values are expressed as a percentage of the pre-1-AS control discharge (=100%). The arrow and dashed vertical indicate the time at which the injections were made. Long lasting increases in resting discharge well above those seen before the administration of 1-AS were evoked by injection of 1 μg cicaprost.

Examination of units tested both with PGE_2 and cicaprost revealed that four units excited only weakly by PGE_2 were excited strongly by cicaprost. A further two units were excited by cicaprost, while PGE_2 had no effect.

Effects of 1-AS on mechanical responsiveness

Injection of 1-AS (50 $mgkg^{-1}$, i.v.), caused a reduction in responsiveness to mechanical stimuli, repeated once every two minutes, in all eleven units examined. Responsiveness began to decline 2 - 26 minutes after administration, and reached a minimum level after 8 - 34 minutes (mean: 21 ± 3.2 mins). The mean minimum discharge per stimulus attained, calculated from values taken at different times following injection, was 50% of pre-injection control. Recovery to higher levels of responsiveness was not seen for observation periods of up to 40 minutes. Figure 7.21 shows pooled data from the responses of the eleven units to 1-AS.

Effects of prostanoids on mechanical responsiveness following 1-AS

The effects of PGE_2 were examined on seven mechanonociceptors whose mechanical responsiveness had been reduced by 1-AS. Injection of PGE_2 (0.03 - 30 μg, i.a.), caused a small increase in responsiveness towards levels seen before administration of 1-AS in four (57%) of these units. There was a mean latency to onset of 2.9 ± 0.7 minutes, and the effect was generally short lived, with a duration of 1 - 8 stimuli (mean: 4 ± 1.7 mins). Dose dependency of the response was not seen in any of the

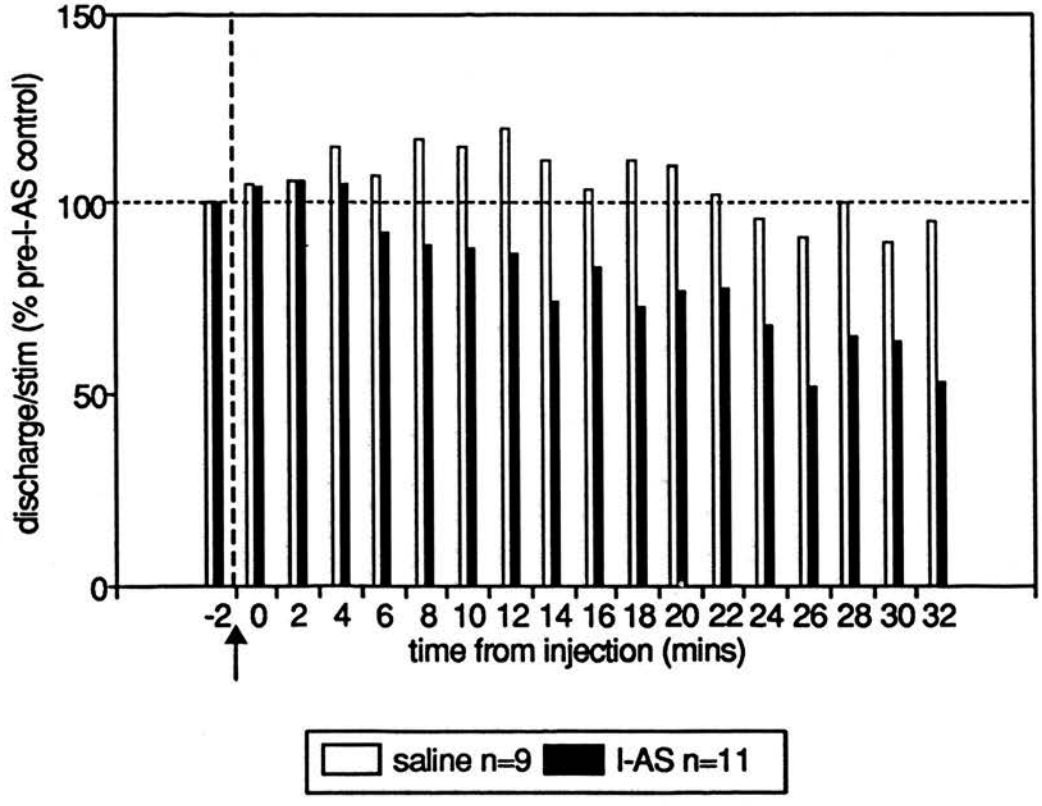


Fig. 7.21 Pooled data showing the depressant effects of l-AS on mechanically evoked discharge from articular mechanonociceptors. Each bar represents the mean response to mechanical stimuli of 2 s duration applied once every 2 mins before and after i.v. injection of l-AS (50 $mgkg^{-1}$, ASA equivalent). Responses before and after injection of saline are also shown. The arrow and dashed vertical line show the time at which injections of l-AS were made. Mean values are significantly ($p < 0.05$, Wilcoxon) lower than corresponding values following saline injection, from 2 mins after injection of l-AS.

four units examined for this relationship. Maximum responses were obtained with doses ranging from 0.03 - 3 μg. Pooled data from maximum responses are shown in figure 7.22.

The effects of cicaprost were examined on seven mechanonociceptors whose responsiveness had been reduced by 1-AS. Injection of cicaprost (0.1 - 1 μg, i.a), caused a large and sustained increase in mechanical responsiveness in six (86%) of these units. This effect had a latency to onset of 3 ± 0.4 minutes, and a duration of 1 - 24 stimuli (mean: 19 ± 4.4 mins). Dose dependency of the response was seen in all four units examined for this relationship, as shown in figure 7.23. Maximum responses were obtained with doses ranging from 0.1 - 1 μg, and pooled data from these responses are shown in figure 7.23.

Consideration of units tested with both PGE_2 and cicaprost revealed that one unit, affected only weakly by PGE_2, displayed a marked increase in responsiveness following cicaprost. A further two units gave a large response to cicaprost, while being unaffected by PGE_2.

2.2.2.2 Electrophysiology in vitro

Examination of the effects of 1-AS were carried out in three experiments, from which three mechanonociceptors were identified. Two units were further examined for the effects of cicaprost following 1-AS. Mechanosensitive units had action potential spike shape characteristics similar to those of identified C fibre units. An an irregular ongoing resting discharge (mean: 1.4 ± 0.3 i.p.s.) was present for two of the three units.

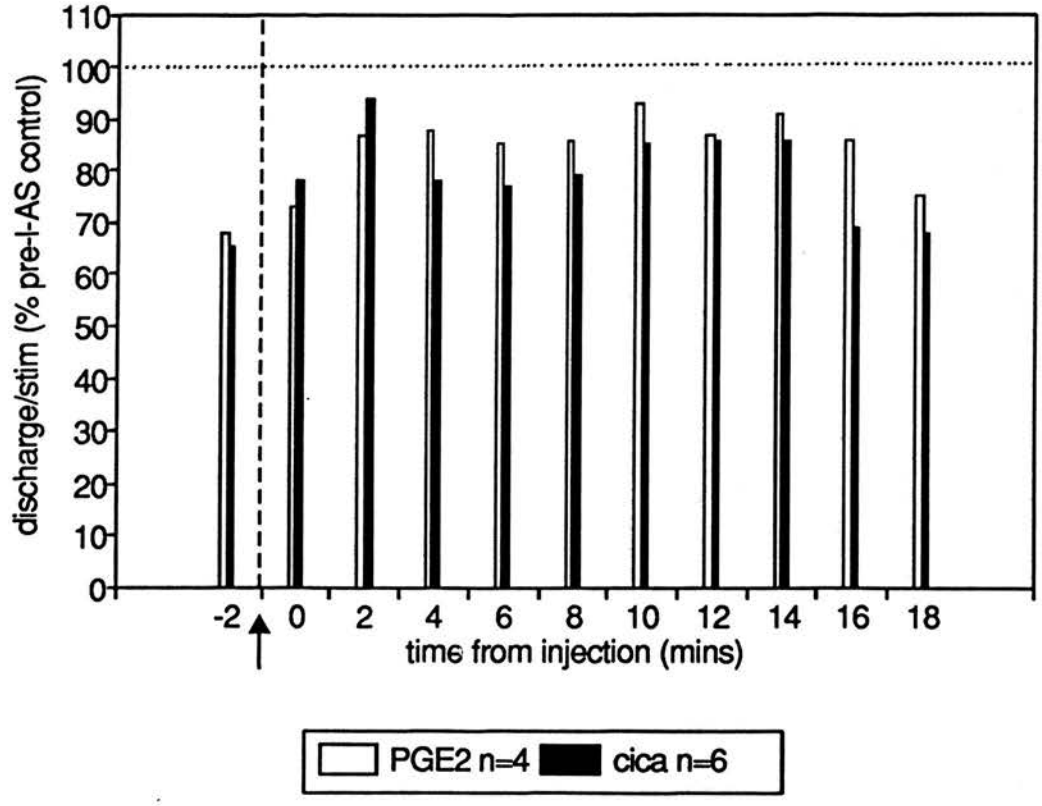


Fig. 7.22 Pooled data showing the maximum sensitizing effect of PGE_2 or cicaprost on the mechanically evoked discharge from articular mechanonociceptors following treatment with 1-AS. Each bar represents the mean response to mechanical stimuli of 2 s duration applied once every 2 mins before and after injection of PGE_2 (0.03 - 3 μg, i.a.) or cicaprost (0.1 - 1 μg, i.a.). Values are expressed as a percentage of pre-1-AS control. PGE_2 caused a sensitization to mechanical stimuli in only 57% of units, whereas cicaprost sensitized 86%. Only those values for responsive units are shown. The arrow and dashed vertical line show the time at which injections were made.

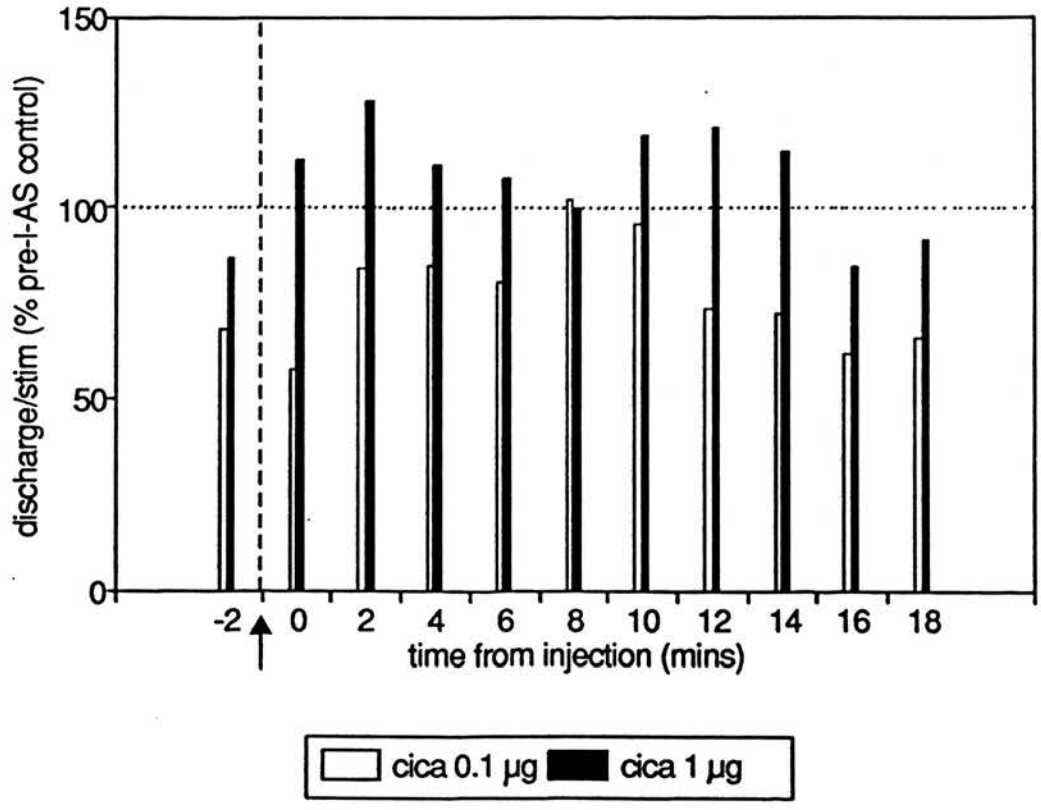


Fig. 7.23 Dose-dependent sensitizing effects of cicaprost on mechanically evoked discharge from three articular mechanonociceptors following treatment with l-AS. Each bar represents the mean response to mechanical stimuli of 2 s duration applied once every 2 mins before and after injection of cicaprost. Values are expressed as a percentage of pre-l-AS control (=100%). The arrow and dashed vertical line indicate the time at which injections of cicaprost were made. Increasing the dose of cicaprost from 0.1 μg to 1 μg increased both the duration and amplitude of the response.

Effects of 1-AS on resting discharge

Combined perfusion and superfusion of 1-AS (Krebs solution containing 100 mgl^{-1}, 1-AS, equivalent to 50 mgl^{-1} ASA) caused a reduction in resting discharge in both of the units examined. A reduction in the occurrence of irregular bursting activity was the most obvious effect. Afferent discharge began to decline at 1 and 5 minutes after the start of the infusion, and reached a minimum level after a delay of 12 and 23 minutes. The mean minimum discharge level attained was 10% of pre-1-AS control. Recovery to higher levels of resting discharge was not seen for recording periods of 40 minutes. Figure 7.24 illustrates a summary of the responses of the two units to 1-AS.

Effects of cicaprost on resting discharge following 1-AS

The effects of cicaprost were examined on two mechanoreceptors whose resting activity had been reduced by 1-AS. Injection of cicaprost (0.01 - 1 μg, i.a.) evoked an afferent discharge in both of the units tested. This effect had a latency to onset of 1 - 5 minutes, and lasted for 3 - 9 minutes. Figure 7.25 illustrates the dose-dependent effect of cicaprost on the one unit tested in this way.

Effects of 1-AS on mechanical responsiveness

Addition of 1-AS (50 mgl^{-1}, ASA equivalent) to the Krebs medium perfusing and superfusion the tissues, reduced mechanically evoked discharge (stimuli applied once every two minutes) in all three units

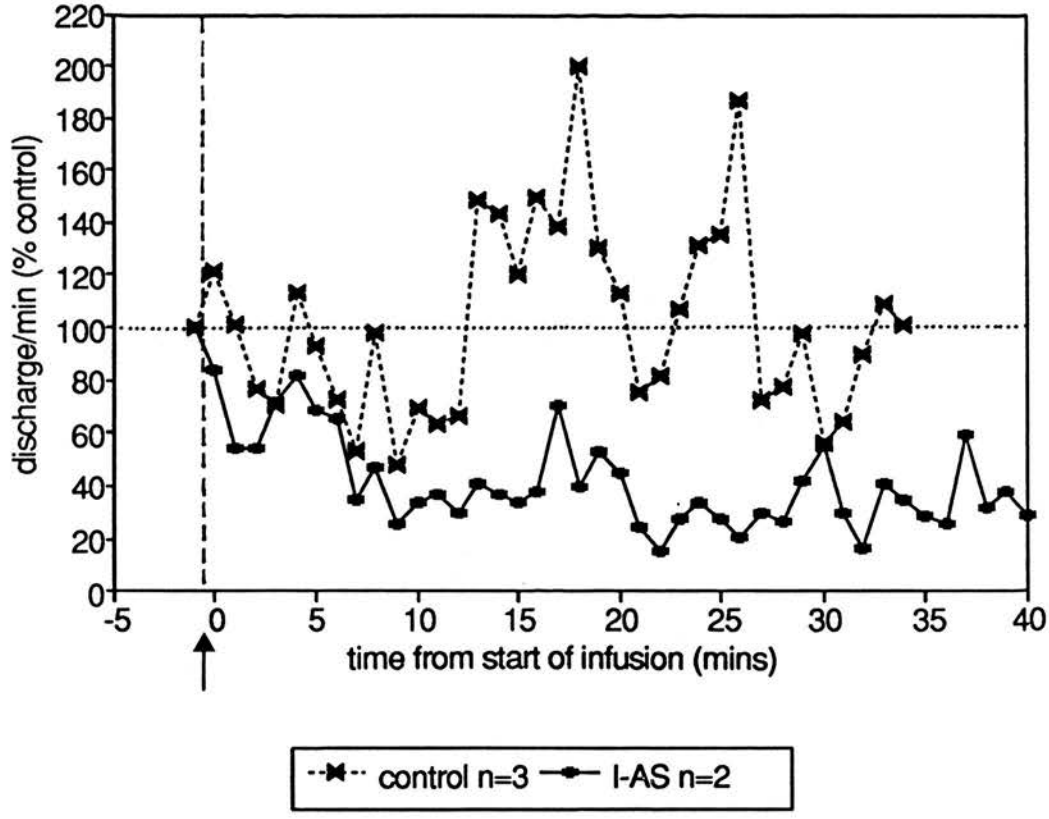


Fig. 7.24 Pooled data showing the depressant effects of l-AS on the resting discharge of articular mechanonociceptors <u>in vitro</u>. Each point on the graph represents the mean discharge recorded over a 60 s period, and is expressed as a percentage of the pre-injection control (=100%). The arrow and vertical dotted line indicate the time at which l-AS was administered. Shown are the effects of l-AS (50 mgl^{-1}, ASA equivalent), or standard Krebs medium on afferent discharge. Mechanonociceptors from arthritic joints displayed a high level of bursting activity, which is reflected in the variability in the discharge obtained when perfusing with standard Krebs medium. The main action of l-AS was to reduce the bursting activity of mechanonociceptors seen in these joints.

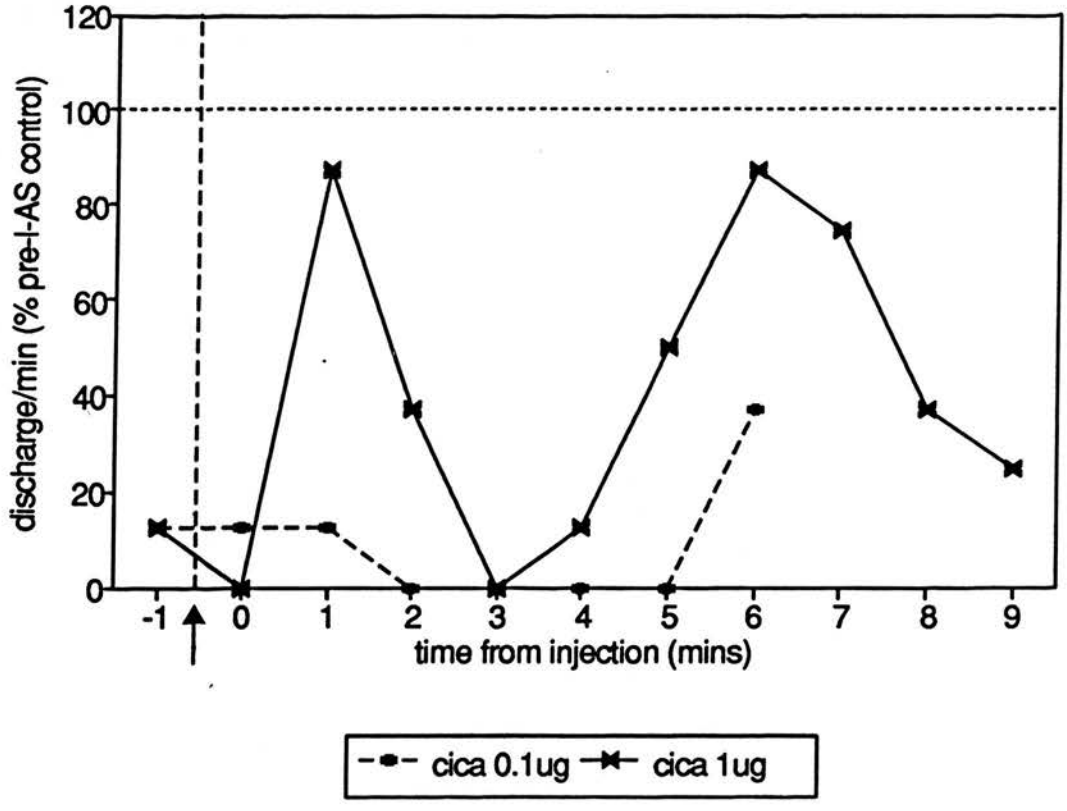


Fig. 7.25 Dose-dependent excitatory effects of cicaprost on the spontaneous discharge of articular mechanonociceptors following treatment with l-AS <u>in vitro</u>. Each point on the graph represents the mean discharge recorded over a 60s period, and is expressed as a percentage of the pre-l-AS control (=100%). The arrow and vertical dotted line indicate the time at which injections were made.examined.

Responsiveness began to decline 2 - 18 minutes after the addition of 1-AS, and reached a minimum level after a delay of 10 - 32 minutes (mean: 24.7 ± 7.3 mins). The mean minumum discharge attained per stimulus, calculated from values taken at different times following administration of 1-AS, was 7% of pre-1-AS control. Recovery to higher levels of responsiveness was not seen for observation periods of 40 minutes. Figure 7.26 illustrates a summary of the responses of the three units to 1-AS.

Effect of cicaprost on mechanical responsiveness following 1-AS

The effects of cicaprost were examined on two units whose mechanical responsiveness had been reduced by 1-AS. Injection of cicaprost (0.01 - 1 μg, i.a.), caused an increase in responsiveness towards levels seen before administration of 1-AS in the one unit on which the highest dose of 1 μg was tested. Figure 7.27 illustrates the dose-dependent effect of cicaprost on this unit.

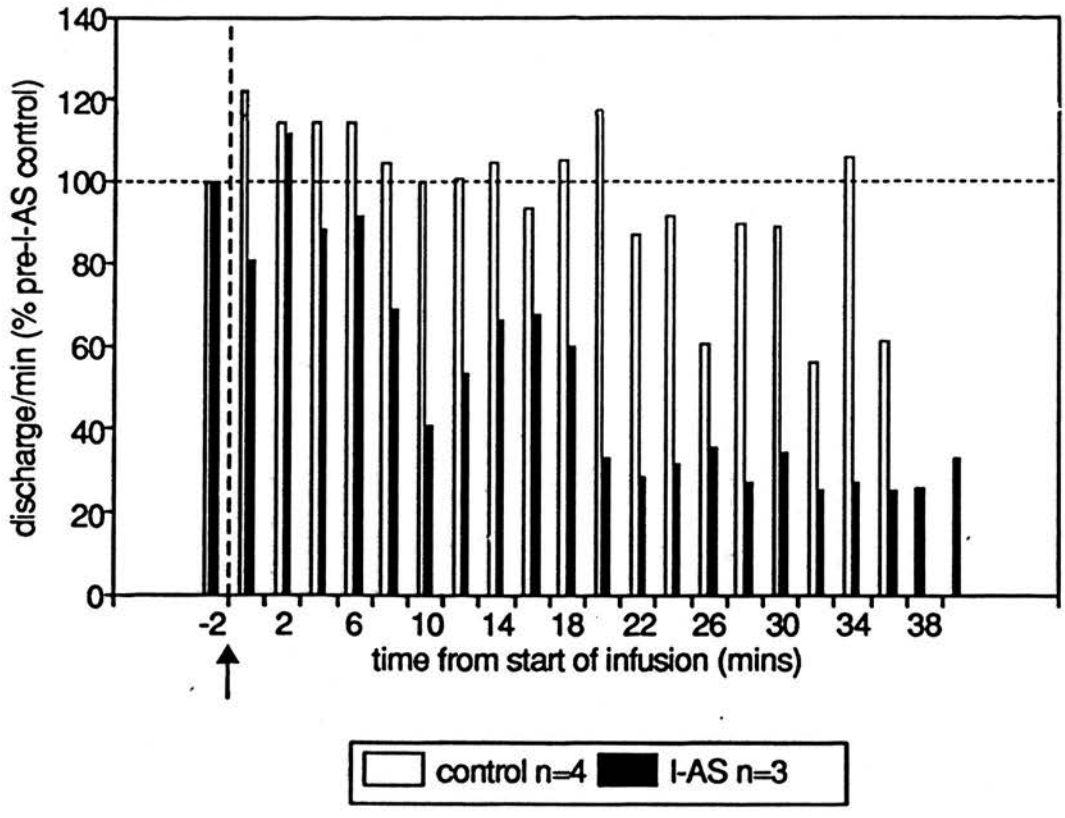


Fig. 7.26 Pooled data showing the depressant effects of l-AS on mechanically evoked activity of articular mechanonociceptors <u>in vitro</u>. Each bar represents the mean response to mechanical stimuli of 2 s duration applied once every 2 mins before and after addition of l-AS (50 mgl^{-1} ASA equivalent) to the Krebs medium. Values are expressed as a percentage of the pre-l-AS control response (=100%). Mechanically evoked responses before and after injection of saline are also shown. The arrow and dashed vertical line show the time at which l-AS was administered.

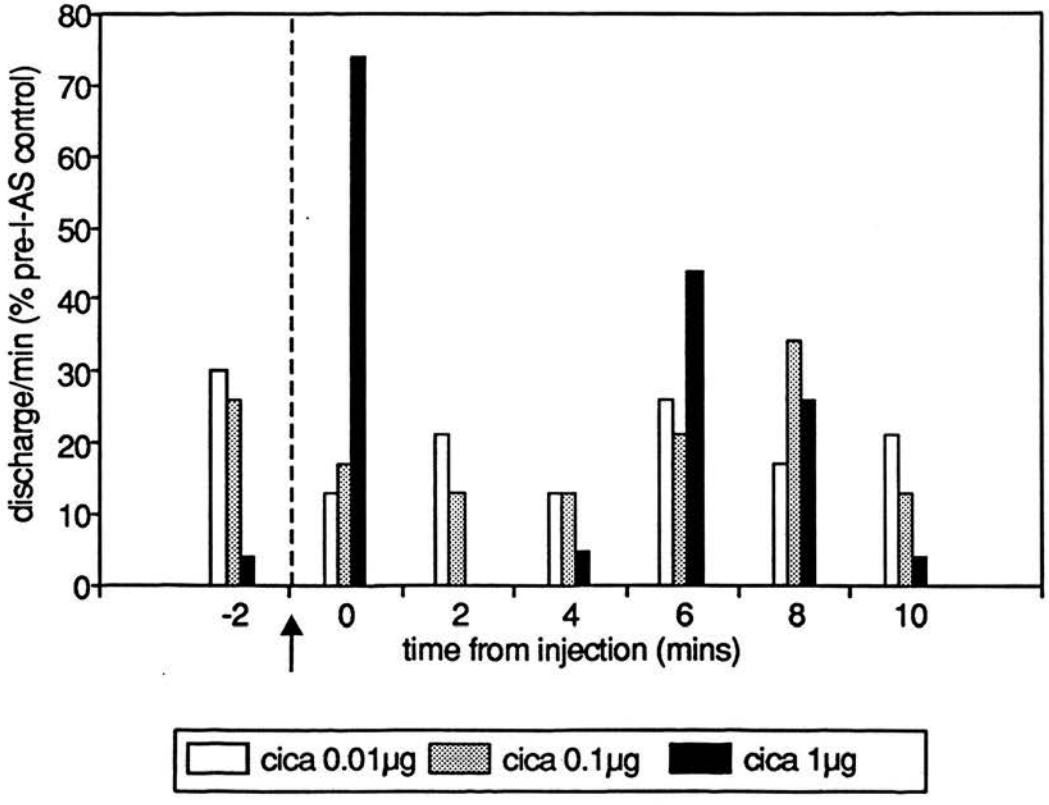


Fig. 7.27 Dose-dependent effects of cicaprost mechanically evoked discharge of an articular mechanonociceptor following treatment with I-AS <u>in vitro</u>. Each bar represents the mean unit response to mechanical stimuli of 2 s duration applied once every 2 mins before and after i.a. injection of cicaprost. The arrow and dashed vertical line show the time at which injections were made. Only the highest dose (1 μg) evoked an increased responsiveness to mechanical stimuli.

7.3 DISCUSSION

The results obtained in the present study show that the prostanoids PGE_2 and PGI_2 have several actions on articular nociceptors. These include excitation, and sensitization to mechanical and chemical stimuli. Both PGI_2 and cicaprost, the highly selective IP-receptor agonist, were more potent than PGE_2 in all of these actions.

7.3.1 Normal joints

7.3.1.1 Receptor excitation

Prostanoid-induced sensitization of articular nociceptors both to mechanical and chemical stimuli described above is consistent with previous findings showing the hyperalgesic and sensitizing properties of the prostanoids in other systems (Ferreira, 1972; Moncada et al., 1975; Handwerker, 1976a,b; Chahl & Iggo, 1977; Tyers & Haywood, 1979; Higgs et al., 1979; Mense, 1981; Petromichelakis & Rood, 1982; Martin et al., 1987). However, the prostanoids have only rarley been shown to produce excitation of nociceptive sensory receptors when given alone. In the skin of the rat, injections of PGE_1 evoked a long lasting (up to 20 mins) discharge in 80% of recordings of afferent activity from the saphenous nerve (Chahl & Iggo, 1977). In cat muscle, PGE_2 (i.a.) evoked a weak, irregular discharge in 46% of C-fibre afferent units (Mense, 1981). Finally, in the cat knee joint, PGE_2 excited approximately 60% of all A-delta and C fibre afferent units (Schaible & Schmidt, 1988b). These effects of PGE_2 in the cat had a latency to onset of 10 - 30

seconds, were of long duration (generally at least 3 mins), and were dose-dependent in the majority of units. In the present study, injections of PGE_2 *in vitro* evoked weak excitation in less than a quarter of mechanonociceptors, and *in vitro* affected none of the mechanonociceptors examined. In contrast to the small numbers of units responding to PGE_2, injection of relatively low doses of PGI_2, *in vivo* excited 80% of the units under study. PGI_2-evoked activity was of long duration, generally lasting for well over 10 minutes. Similar results were obtained *in vivo* and *in vitro* for the highly selective IP-receptor agonist cicaprost. In the present study, the high potency of PGI_2 and cicaprost, the low potency of PGE_2, and the lack of effect with $PGF_{2\alpha}$ or PGD_2 suggests that, in the rat, prostanoid-induced excitation of articular nociceptors is mediated by IP-receptors. The relatively potent excitatory effects of PGE_2 reported by Schaible & Schmidt (1988b) in the cat knee joint may reflect a species difference in the populations of IP- or EP-receptors or present on articular sensory nerves. However, it is difficult to draw any conclusions from this study in the cat as only PGE_2 was used, and no comparison with the effects of other prostanoids can be made. Excitatory effects of PGE_1 reported in rat skin by Chahl & Iggo (1977), may have been mediated by IP-receptors, EP-receptors or both, since this prostanoid has potent agonist effects at both receptor types (see Jones et al., 1984). Studies on the depolarizing effects of the prostanoids on the rabbit or rat isolated vagus nerve (see Section VIII), suggest that populations of EP-receptors present on C fibre sensory nerves differ in these two species.

Examination of the excitatory effects of prostanoids on chemosensitive articular receptors *in vitro* revealed a similar rank order of potency to

that seen for mechanonociceptors. $PGF_{2}\alpha$ and PGD_2 had no effect, while PGE_2 and cicaprost caused excitation, with cicaprost being the more effective. These results support the idea that excitatory prostanoid receptors on rat chemosensitive sensory receptors are mainly of the IP type, but does not exclude the involvement of an EP-receptor in prostanoid-evoked excitation of these units.

In support of the idea that prostanoids may cause pain when given alone are various studies on animals and man. Intra-arterial, intravenous or intramuscular injections of the E-series prostanoids (Bergstrom et al., 1965; Carlson, 1968; Bevergard and Oro, 1969; Karim, 1971; Collier et al. 1972; Gillespie, 1972), PGI_2 (Fitzgerald et al. 1979; O'Grady et al., 1980; Szceklick et al., 1980) or its stable analogue, iloprost (Kaukinen et al., 1984) have been reported to cause pain and headache in man. In the dog intra-arterial injections of PGE_2 into the spleen evokes pseudoaffective response (Moncada et al., 1974), and when injected into the knee joint PGE_2 or PGE_1 cause an incapacitation (Rosenthale et al., 1972). Additionally, intraperitoneal injection of PGE_2 or PGE_1 evokes a writhing response in mice (Collier & Schneider, 1972).

A dissociation between the hyperalgesic effects of low dose PGE_1, and the pain-producing effects of high dose PGE_1 was demonstrated by Ferreira (1972), who injected the prostanoid intra-dermally in man. These effects may reflect an action of PGE_1 at two different prostanoid receptors, or may be related to an increase in the activation of the same system at higher doses.

7.3.1.2 Mechanical sensitization

Prostanoid-induced sensitization of sensory receptors to mechanical stimuli has been reported previously in rat skin for PGE_2 on A-delta and C fibre nociceptors (Martin et al., 1987), and for PGE_1 on moderate pressure A-delta mechanoreceptors (Pateromichelakis & Rood, 1982). In the cat knee joint, sensitization to joint movement has also been reported for PGE_2 on articular sensory receptors with A-delta and, to a lesser extent, C fibre afferents (Schaible & Schmidt, 1988b). In the present study, PGI_2 and cicaprost both sensitized nearly double the number of units affected by PGE_2. In contrast to the long duration effects of PGI_2 or cicaprost, PGE_2-induced sensitization was generally short-lived and showed no dose-dependency. These results suggest that these effects are predominantly mediated by IP-receptors.

Of further interest is the finding that PGE_2-induced effects on the mechanical responsiveness of the units under study had two components: at low doses sensitization occurred in just under half the units, whereas at higher doses a marked reduction in mechanical sensitivity was seen. This observation suggests that PGE_2 may be acting at two different prostanoid receptors to produce these effects. The presence of an EP-receptor mediating mechanical sensitization is supported by the finding that PGI_2-induced effects were of greater amplitude and longer duration than those of cicaprost. PGE_2-induced depression of mechanical responsiveness may be mediated by an EP-receptor subtype or perhaps by a receptor for a different endogenous prostanoid.

Hyperalgesia to mechanical stimuli has been reported by other workers for PGE_1, PGE_2 or PGI_2 in the paw of the rat (Willis & Cornelsen, 1973;

Ferreira et al., 1978; Tyers & Haywood, 1979). Following their injection into the knee joint of the dog, both PGE_2 and PGI_2 cause incapacitation (Ferreira et. al., 1978). Ferriera (1972) stated that the hyperalgesic effects of PGI_2 were more rapid in onset and of shorter duration than than those of PGE_2, which took up to thirty minutes to develop, and lasted for several hours. It is difficult to compare this finding with those of the present study since the method of administration and assessment of effects was somewhat different. Since injection into the paw results in the localization of the drug in that area, the known pharmacokinetics of these prostanoids may explain why the effects of PGI_2, which is hydrolysed very rapidly to 6-keto-$PGF_{1\alpha}$ at physiological pH (Cho & Allen, 1978), are of short duration, whereas those of the relatively stable PGE_2 (Ferreira, 1979) are prolonged. The long latency to onset of PGE_2-induced hyperalgesia may be related to the time taken for the drug to diffuse throughout the tissues, and to reach sufficiently high concentrations to affect a significant number of nociceptors.

7.3.1.3 Interactions with bradykinin

The sensitizing properties of the prostanoids on bradykinin-induced excitation of sensory receptors has been demonstrated previously in the skin of the rat for PGE_1 (Chahl & Iggo, 1977) or PGE_2 (Handwerker, 1976), in cat muscle for PGE_2 (Mense, 1981), in canine testicular tissues for PGE_2 or PGI_2 (Kumazawa et al, 1987), and in the cat knee joint for PGE_2 (Schaible & Schmidt, 1988b). In the present study, both *in vivo* and *in vitro*, the most significant potentiation of bradykinin-

induced excitation was seen with cicaprost. PGE_2 generally caused only a weak enhancement of responses to bradykinin. PGI_2 evoked a sustained increase in discharge from most units when given alone at the lowest dose used in vivo. These results suggest that prostanoid-induced potentiation of bradykinin-evoked excitation is mediated by an IP-receptor. Comparison of the potentiating effects of PGE_2 and PGI_2 on the response of canine testicular C-polymodal nociceptors to bradykinin, also showed PGI_2 to be more effective, there being a ten fold difference in the potency of the two prostanoids (Kumazawa et al., 1987).

Bradykinin-induced sensitization to mechanical stimuli was also found to be markedly potentiated by PGE_2 or PGI_2, and by cicaprost. In this respect the effects of PGE_2 are particularly interesting since an effective dose of this prostanoid, which on its own caused a depression of mechanical responsiveness, evoked a sensitization when combined with bradykinin. These effects may be mediated by two different prostanoid receptors, one causing depression and the other sensitization, with the combined sensitizing effects of PGE_2 and bradykinin overcoming the depressant effect of high dose PGE_2.

The ability of prostanoids to potentiate the algesic effects of bradykinin have been demonstrated in a number of different models. Injection of bradykinin into the spleen of the dog or cat induced a pseudoaffective response which was potentiated by PGE_2 or PGE_1 (Ferreira, 1972; Tyers and Haywood, 1979), with PGE_1 being over a thousand times more potent than PGE_2 (Tyers and Haywood, 1979). In the rabbit isolated ear preparation, the reflex fall in arterial blood pressure induced by bradykinin was potentiated by PGE_2, PGE_1 or PGI_2 (Lembeck et al., 1976; Juan, 1979). In this model PGI_2 and PGE_1 were

approximately thirty times more potent than PGE_2 (Juan, 1979). These findings are in agreement with those of the present study, and support the involvement of IP-receptors in prostanoid-induced potentiation responses to bradykinin.

$PGF_2\alpha$-induced depression of the potentiating effects of PGE_1 on responses to bradykinin by been reported in the rabbit isolated ear preparation (Juan & Lembeck, 1977). These results suggest that in the present study the depressant effects of PGE_2 on responsiveness to mechanical stimuli may be mediated via the same receptor type as that mediating the effects of $PGF_2\alpha$ in the rabbit ear. In the present study the effects of $PGF_2\alpha$ on the responsiveness of articular mechanonociceptors to mechanical stimuli was not examined, and should probably be considered in future investigations.

7.3.2 Arthritic joints

7.3.2.1 Effects of lysine acetylsalicylate (l-AS)

The results described above confirm those obtained by Guilbaud et al. (1985) in rats with adjuvant polyarthritis, and establish that l-AS causes a reversible depression of mechanical responsiveness and ongoing discharge in articular mechanonociceptors from chronically inflamed rat ankle joints. It was previously shown that l-AS has no effect on similar, although less sensitive, mechanoreceptors in normal joints (Guilbaud et al., 1985). The time course of the depressant effect seen here is similar to that reported for the effect of ASA on the excitation of muscle nociceptive afferents by bradykinin in the cat (Mense, 1982),

and also with that described for the effects of ASA or indomethacin on articular nociceptors from the acutely inflamed cat knee joint (Heppelman et al., 1986). These findings suggest that these drugs operate via a peripheral site of action.

The doses of l-AS used here are in the same range as those commonly used in studies to evaluate the analgesic action of ASA (Hirata M., 1966; Blane, 1967; Abe et al., 1971; O'Dea, 1975; Deraedt et al., 1976; Gouret et al., 1976; Vinegar, 1976; Inoki, 1977), and the onset and duration of the depressant effect is similar to that of analgesia produced by ASA in the dog (O'Dea et al., 1975), and in man (Kantor et al., 1966; Mehlisch, 1983).

7.3.2.2 Effects of the prostanoids after treatment with l-AS

It has been established both <u>in vitro</u> (Vane, 1971; Ferreira & Vane, 1974; Roth et al., 1975; Sturge et al., 1978; Van der Ouderas et al., 1980; Salmon et al., 1983), and <u>in vivo</u> (Ferreira and Vane, 1974; Moncada et al., 1975; Fitzpatrick & Wynalda, 1976), that acetylsalicylate inhibits prostanoid synthesis, and is generally considered to exert its analgesic effects via the resulting reduction in tissue prostanoid content (Collier et al., 1972; Ferreira and Vane, 1974; Moncada et al., 1975). From the time constants for the effects of l-AS on the responsiveness of articular receptors Guilbaud et al. (1985) concluded that a cyclooxygenase metabolite with a half life comparable to that of PGI_2 was responsible for the sensitization of mechanoreceptors in arthritic joints. However, several enzymes other than prostanoid cyclooxygenase may be affected by aspirin-like drugs

(Lembeck & Juan, 1974; Tolman & Partride, 1975; Kuehl and Egan, 1980; Brune, 1982; 1983), and salicylate has been found to have direct actions on neuronal membranes (Levitan & Barker, 1972a,b,c; Neto & Narahashi, 1976; Neto, 1980).

In the present study it was demonstrated that following depression of afferent discharge by 1-AS exogenously administered PGE_2 or cicaprost could evoke increases in activity towards those levels seen before treatment with 1-AS, suggesting that these effects of 1-AS are mediated via the inhibition of endogenous prostanoid production. Cicaprost was considerably more effective than PGE_2 both for mechanical responsiveness and ongoing discharge, supporting the idea that PGI_2 is the major endogenous prostanoid responsible for nociceptor sensitization during inflammation.

In the acutely inflamed knee joint of the cat, the depressant effects of ASA or indomethacin, were partially reversed by PGE_2 in all units for ongoing discharge, and in 67% of units for sensitivity to movement (Heppelmann et al., 1986). These effects were of longer duration than those seen in the present study for PGE_2. Although species differences may account for the long lasting effects of PGE_2 in the cat, without a comparison with other prostanoids it is impossible to determine whether or not these are EP-receptor mediated effects.

Following treatment with 1-AS in arthritic joints, responses to cicaprost lasted on average for over twice as long as those seen in normals. Increased sensitivity to cicaprost seen in arthritic joints may be produced as a result of the actions of other inflammatory mediators such as bradykinin, 5-HT (see Sections V and VI), or lypoxygenase metabolites of arachidonic acid. LTB_4, for example, has been shown to

sensitize mechanonociceptors in the rat skin, probably via the release of 8(R),15(S) dihydroxyeicosatetraenoic acid (8(R),15(S)diHETE) from polymorphonuclear leukocytes (Martin et al., 1987).

The reduction both in mechanonociceptor responsiveness and in resting discharge caused by 1-AS *in vitro* was much greater than that seen *in vivo*. This result suggests that factors not present *in vitro* may be responsible for the residual mechanoreceptor sensitization and ongoing activity seen following 1-AS *in vivo*. These additional factors may be blood borne or may have been washed out of the tissues as a result of the perfusion process. The short-lived response evoked by cicaprost *in vitro* following 1-AS also suggest that components present *in vivo* may be potentiating the effects of cicaprost in the arthritic joint. Mediators likely to be involved include 5-HT and bradykinin (see Sections V and VI respectively).

7.3.2 Mechanisms of action

Since all the effects of the prostanoids which were seen *in vivo* could also be demonstrated *in vitro*, these are probably directly mediated effects and do not involve the release of secondary blood borne mediators or spinal reflex activity. Support for a direct action mediated by prostanoid receptors associated with sensory nerves is provided by studies showing effects on isolated neurones *in vitro*. On rat sensory neurones in culture application of PGE_2 causes these cells to fire action potentials (Baccaglini & Hogan, 1983). In rabbit C-type nodose ganglion neurones PGE_1, PGE_2 and PGD_2 inhibit a slow after-hyperpolarization via the blockade of a Ca^{2+}-dependent K^+ conductance

(Fowler et al, 1985a,b; Weinreich & Wonderlin, 1987). This effect of the prostanoids was mimicked by forskolin suggesting that inhibition of the Ca^{2+}-depedent K^+ conductance is mediated via cAMP (Weinreich & Wonderin, 1987). Prostanoid-induced depolarization of the rabbit isolated vagus nerve in vitro (see Section IV) could be mimicked by forskolin, and also by 8-bromo cAMP, suggesting that depolarization is mediated via cAMP. These findings support the idea that prostanoids affect the excitability of nociceptive sensory neurones via prostanoid receptors located on these neurones.

7.4 CONCLUSIONS

The results described above provide strong evidence for the involvement of IP-receptors in the excitatory and sensitizing effects of exogenously administered prostanoids. There is also some support for the existence EP-receptor mediated sensitizing effects, and further studies using selective EP receptor agonists (see Section XIII) would be of use in determining which EP-receptor subtype(s) may be involved. Results from arthritic joints suggest that PGI_2 is the major endogenous prostanoid responsible for the sensitization of articular mechanonociceptors from chronically inflamed ankle joints in the rat. In support of a role for PGI_2 in human joint disease, an extensive study examining the prostanoid content of synovial fluid from inflamed joints found that the stable hydration product of PGI_2, 6-oxo-$PGF_{1\alpha}$, was in greater abundance than any of the other prostanoids (Brodie et al., 1980). As detailed above, administration of 1-AS did not result in the complete suppression of

ongoing activity and mechanical responsiveness in arthritic joints, and it is likely that inflammatory mediators other than the prostanoids are involved in maintained nociceptor sensitization in these joints.

SECTION VIII

PROSTANOID-INDUCED DEPOLARIZATION OF SENSORY AND MOTOR NERVES FROM THE RABBIT AND RAT

SECTION VIII

PROSTANOID-INDUCED DEPOLARIZATION OF SENSORY AND MOTOR NERVES FROM THE RABBIT AND RAT

8.1 INTRODUCTION

The hyperalgesic actions of the prostanoids, particularly those of the E-series, are well documented (Ferreira, 1972; Moncada et al., 1975; Ferreira et al., 1978; Ferreira & Nakamura, 1979a; Tyers & Haywood, 1979; Higgs et al., 1981; Higgs & Moncada, 1983), and PGE_2, PGE_1 and PGI_2 have been shown to sensitize nociceptive sensory receptors to a range of mechanical (Martin et al., 1987), and chemical (Handwerker, 1976; Chahl & Iggo, 1977; Mense, 1981; Yanigasawa et al., 1986; Kumazawa et al., 1987; Schaible and Schmidt, 1988b) stimuli. Furthermore, the prostanoids have been demonstrated to have direct effects on isolated sensory neurones, including the induction of action potential generation in rat sensory neurones in culture (Baccaglini & Hogan, 1983), and the blockade of a Ca^{2+}-dependent after-hyperpolarization in neurones of the rabbit nodose ganglion (Fowler et al., 1985a,b; Weinreich & Wonderlin, 1987).

It has recently been shown that prostanoids can cause dose-related depolarization of the rat isolated vagus nerve *in vitro* (Poll et al., personal communication). Using the naturally occurring prostanoids, the selective IP-receptor agonists, cicaprost and iloprost, and the

selective TXA_2 agonist, U46619, the following rank order of potency was obtained

cicaprost > iloprost > PGI_2 > PGE_1 > PGE_2 > $PGF_{2\alpha}$ > U46619 > PGD_2

These results indicate that in this preparation prostanoid-induced depolarization is mediated predominantly by IP-receptors.

In the present study I have examined prostanoid-induced effects on the rat isolated saphenous and sciatic nerves in order to determine which prostanoid receptors are present on these predominantly sensory and motor nerves of the rat. A mixed nerve, the tibial nerve, was also used in this study. Additionally, a separate series of experiments, using the rabbit isolated vagus nerve, were carried out to compare prostanoid-induced effects on sensory nerves across species. The role played by cAMP in prostanoid-induced effects were also examined in this preparation using the non-hydrolysable analogue of cAMP, 8-bromo cAMP, the adenylate cyclase stimulant forskolin, and the phosphodiesterase inhibitor IBMX.

8.2 RESULTS

The rat and rabbit isolated nerve preparations used in these experiments are described in Section II. Application of the naturally occurring prostanoids and their synthetic mimetics caused a depolarization in all the isolated nerve preparations used. The rabbit isolated vagus nerve was depolarized most consistently, responses showing clear

concentration-dependency. In contrast, although depolarizations were obtained with the rat isolated nerve preparations, they were weak and inconsistent.

8.2.1 Rabbit vagus nerve

8.2.1.1 Naturally occurring prostanoids

PGE_2 (n=10), PGE_1 (n=6) and PGI_2 (n=10) all evoked depolarization of the rabbit isolated vagus nerve in a concentration-dependent manner in the concentration range 1×10^{-9} - 3×10^{-6} M (fig. 8.1). Neither PGD_2 (n=5) or $PGF_{2\alpha}$ (n=3) produced significant effects in concentrations up to 3×10^{-6} M. Construction of log concentration-effect curves (figs. 8.2 - 8.4), revealed that PGE_2 and PGI_2 were of similar potency. A summary of the EC50 values for individual experiments is illustrated in figure 8.8. Table 8.1 shows the EC50 values for each of the prostanoids tested. Mean values were calculated from individual experiments as described in Section II. The most potent natural prostanoid was PGE_1, with an EC50 value of 16 ± 3 nM. Thus, the following rank order of potency was obtained

$$PGE_1 > PGI_2 > PGE_2 >> PGD_2 = PGF_{2\alpha} = 0$$

The mean log concentration-effect curves for PGE_1 and PGI_2 were approximately parallel, however, that for PGE_2 was notably shallower (fig. 8.7).

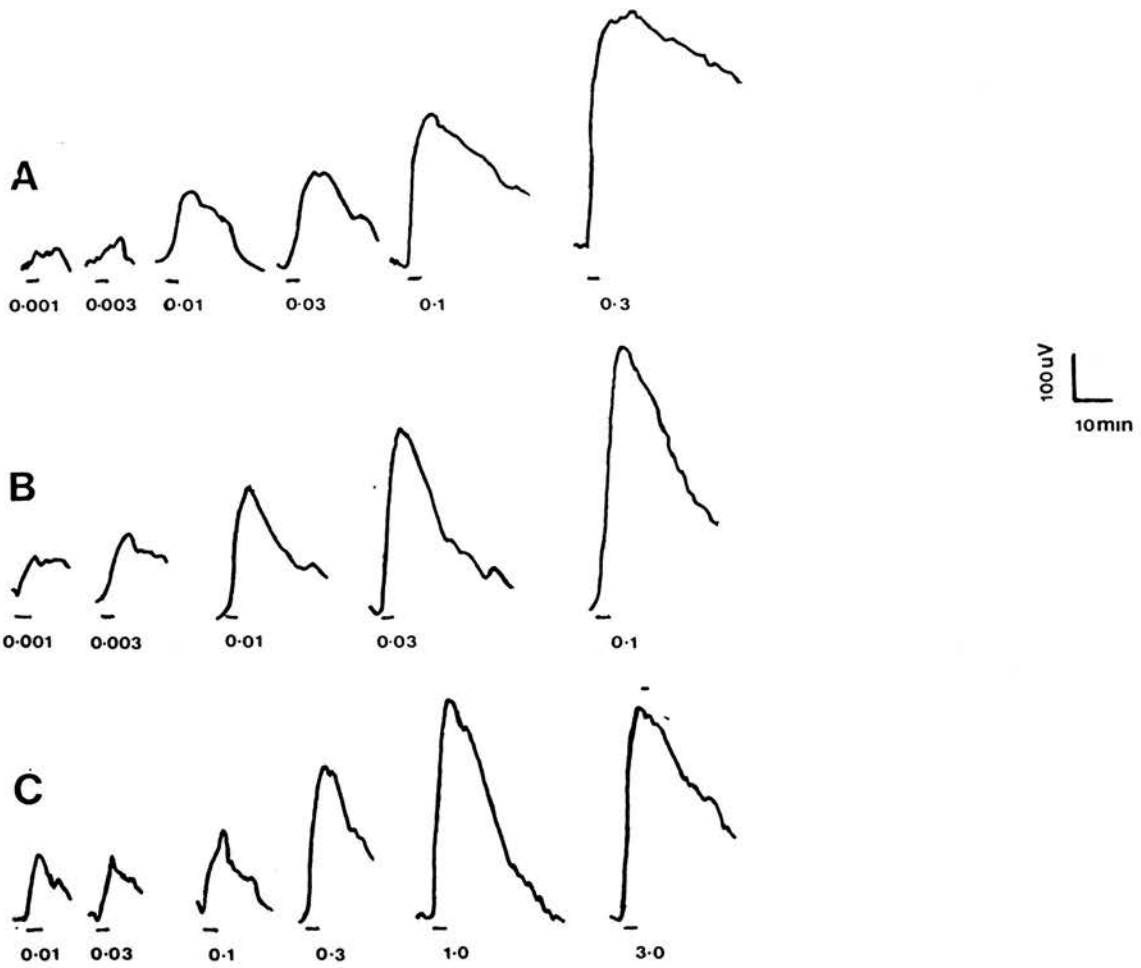


Figure 8.1 Rabbit isolated vagus nerve. Discontinuous record of the effects of (A) PGI_2, (B) PGE_1 and (C) PGE_2 on individual vagus nerve preparations. Upwards direction indicates depolarization: the solid bar under each response shows the appropriate duration of the agonist application (3 mins), with corresponding concentrations of drug given in μM.

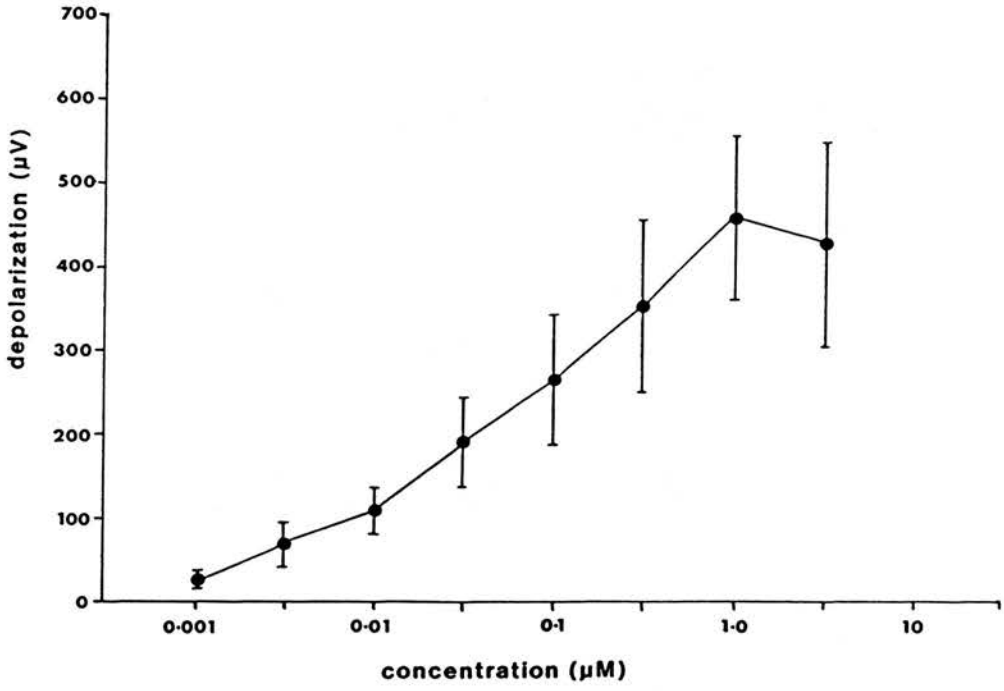


Figure 8.2 Rabbit isolated vagus nerve. Log concentration-effect curve for PGE_2-evoked depolarization. Each point represents the mean ± s.e.m. depolarization evoked by a given concentration of PGE_2 for 10 determinations. Drugs were applied for a period of 3 mins, and tissues were allowed to repolarize fully between applications.

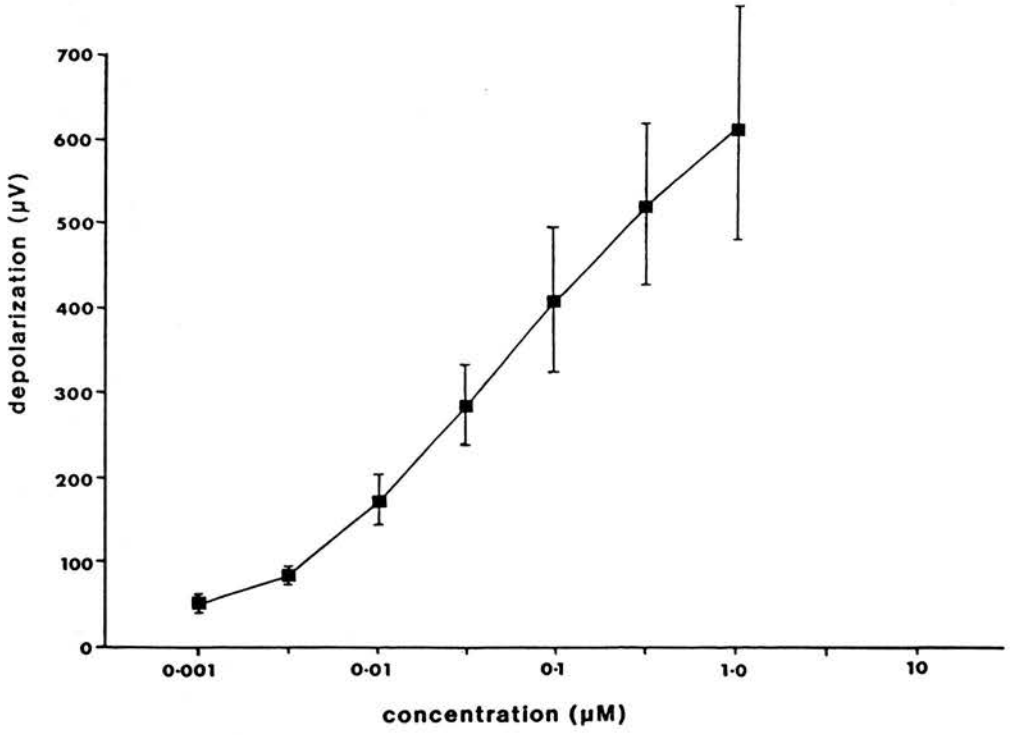


Figure 8.3 Rabbit isolated vagus nerve. Log concentration-effect curve for PGI_2-evoked depolarization. Each point represents the mean ± s.e.m. depolarization evoked by a given concentration of PGI_2 for 10 determinations. Drugs were applied for a period of 3 mins, and tissues were allowed to repolarize fully between applications.

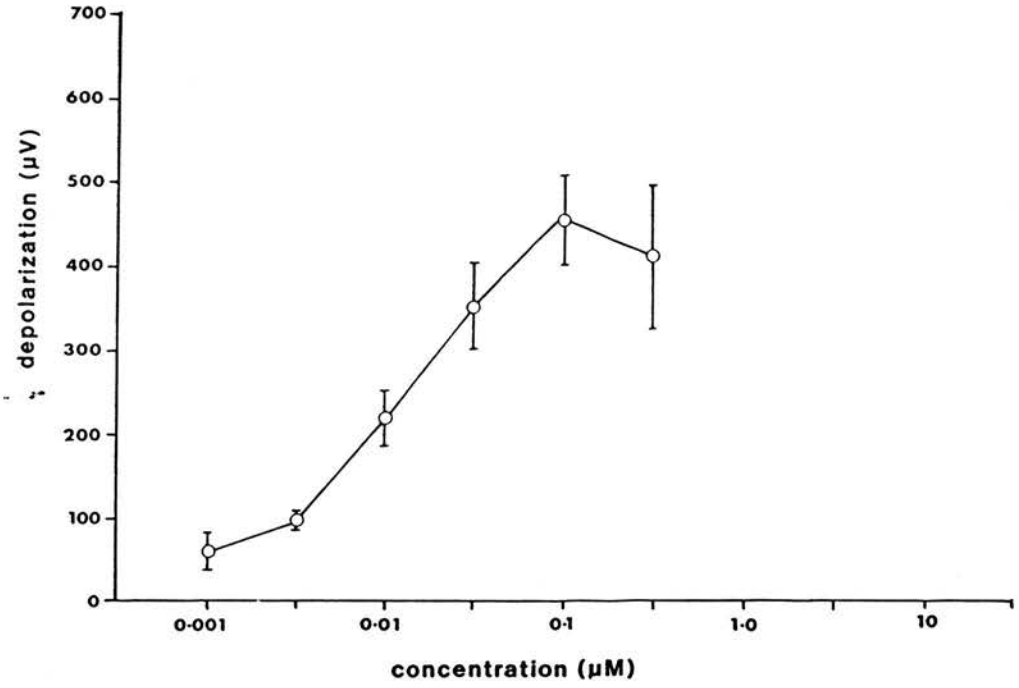


Figure 8.4 Rabbit isolated vagus nerve. Log concentration-effect curve for PGE_1-evoked depolarization. Each point represents the mean ± s.e.m. depolarization evoked by a given concentration of PGE_1 for 6 determinations. Drugs were applied for a period of 3 mins, and tissues were allowed to repolarize fully between applications.

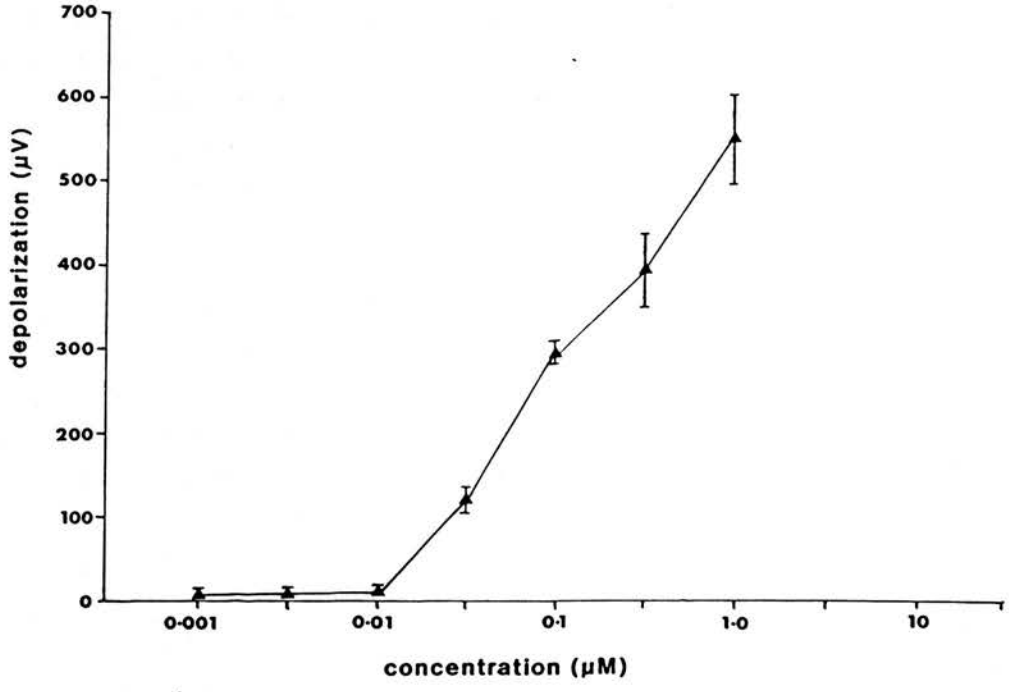


Figure 8.5 Rabbit isolated vagus nerve. Log concentration-effect curve for iloprost-evoked depolarization. Each point represents the mean ± s.e.m. depolarization evoked by a given concentration of iloprost for 3 determinations. Drugs were applied for a period of 3 mins, and tissues were allowed to repolarize fully between applications.

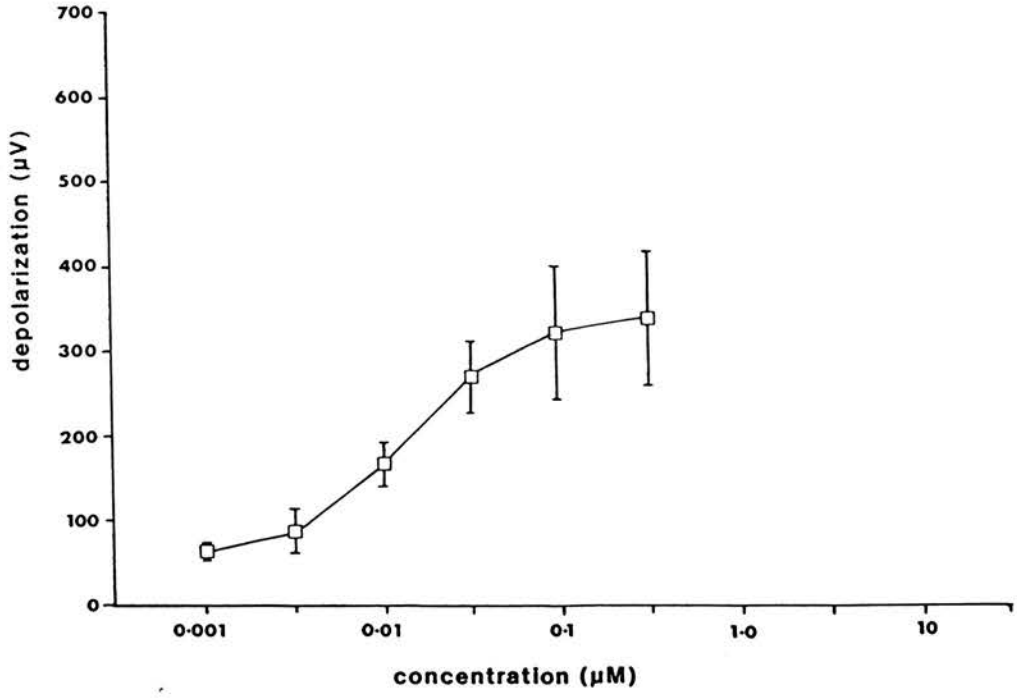


Figure 8.6 Rabbit isolated vagus nerve. Log concentration-effect curve for cicaprost-evoked depolarization. Each point represents the mean ± s.e.m. depolarization evoked by a given concentration of cicaprost for 9 determinations. Drugs were applied for a period of 3 mins, and tissues were allowed to repolarize fully between applications.

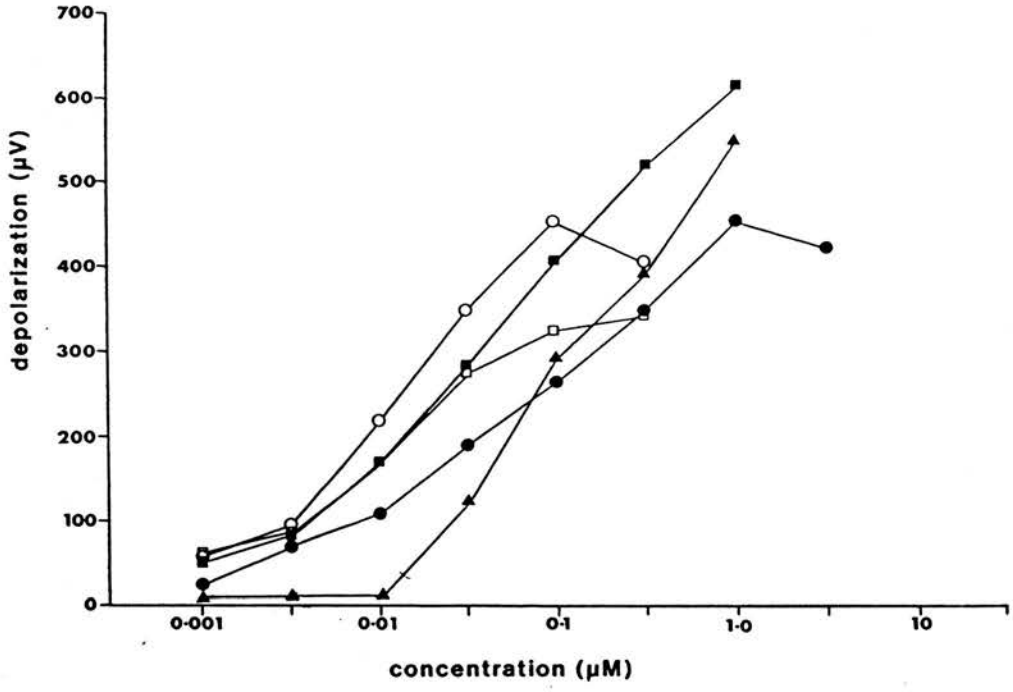


Figure 8.7 Rabbit isolated vagus nerve. Comparison of the effects of a range of prostanoids. Each point represents the mean depolarization evoked by a given concentration of PGE_2 (●, n=10), PGI_2 (■, n=10), PGE_1 (○, n=6), iloprost (▲, n=3) and cicaprost (□, n=9). Drugs were applied for a period of 3 mins, and tissues were allowed to repolarize fully between applications.

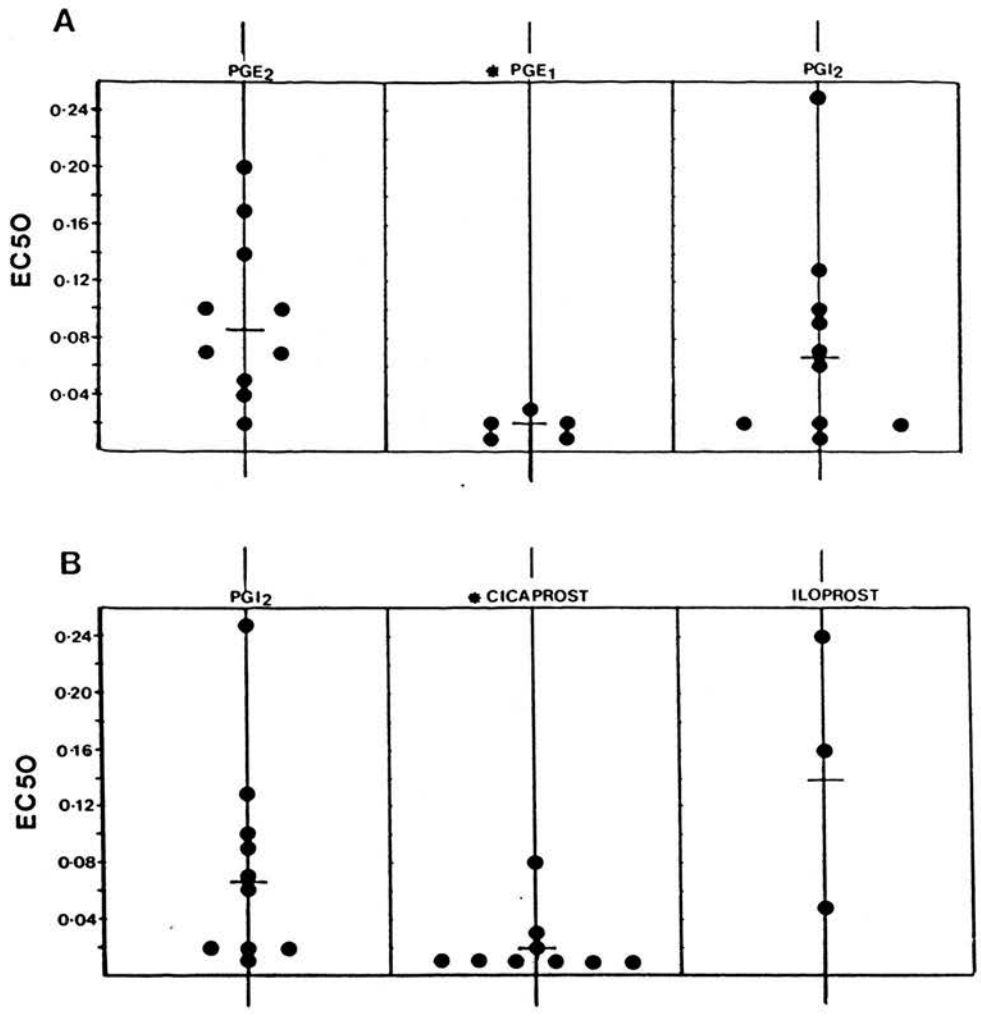


Figure 8.8 Rabbit isolated vagus nerve. Scatter plots illustrating the individual (●) and mean (horizantal bars) EC50 values for a range of prostanoid agonists. (A) Comparison between PGE_2, PGE_1 and PGI_2. (B) Comparison between PGI_2, cicaprost and iloprost. Values are given in μM. Mean values which are significantly different to that for PGI_2 are shown as *, when $p < 0.05$ (Wilcoxon).

Table 8.1 Rabbit isolated vagus nerve. Summary of the EC50 and Emax values for prostanoid-induced depolarization of the rabbit vagus.

prostanoid agonist	EC50 (nM)	Emax (μV)	n
PGE_2	79	496 ± 100	10
PGE_1	16 *	524 ± 90	6
PGI_2	49	632 ± 89	10
$PGF_{2\alpha}$	> 10 000	98 ± 16	3
PGD_2	> 10 000	55 ± 16	5
cicaprost	15 *	357 ± 55	9
iloprost	127	609 ± 123	3
U46619	> 10 000	63 ± 30	3
ICI81008	> 10 000	140 ± 29	3
sulprostone	-	0	2
rioprostil	-	0	3
AH13205	-	0	3

EC50 values expressed as the geometric mean
Emax values expressed as the arthmetic mean ± s.e.m.
* EC50 value significantly smaller than that for PGE_2 ($p < 0.05$, Wilcoxon)

8.2.1.2 Synthetic prostanoids

The selective IP-receptor agonists, cicaprost (n=9) and iloprost (n=3) at concentrations from 1 x 10^{-9} - 3 x 10^{-6} M evoked concentration-dependent depolarization of the rabbit isolated vagus nerve (figs. 8.5 - 8.6). The synthetic $PGF_{2\alpha}$ mimetic, ICI81008 (n=3), the stable TXA_2 mimetic, U46619 (n=3), the selective EP_2-receptor agonist AH13205 (n=3), the EP_2- and EP_3-receptor agonist rioprostil (n=3), and the EP_1- and EP_3-receptor agonist sulprostone (n=2) at concentrations up to 1 x 10^{-5} M did not produce significant effects on the rabbit isolated vagus (see table 8.1). Thus, the following rank order of potency was obtained

cicaprost > iloprost >> ICI81008 = U46619

= sulprostone = rioprostil = AH13205 = 0

A summary of the concentration effect curves obtained for the active naturally occurring prostanoids and synthetic agonists is illustrated in figure 8.7.

8.2.1.3 Desensitization experiments

To examine the possibility that desensitization of responses to the prostanoids developed, sequential log concentration-effect curves were constructed using graded low to high concentrations or high to low concentrations of drug (see Section II). Log concentration-effect curves were constructed for PGE_2 (n=3), PGE_1 (n=3), PGI_2 (n=3) and cicaprost (n=4). Results are summarized in figure 8.8. Mean EC50 values for PGE_2,

PGE_1, PGI_2 and Iloprost, calculated using values from individual experiments, are shown in table 8.2. EC50 values obtained for PGE_1, PGI_2, and cicaprost were not significantly different (Students T-Test) when high to low concentration protocols were used rather than the standard low to high concentration protocols. Analysis of EC50 values for PGE_2 showed a significant (Students t-test, $p < 0.05$) shift of the curve to the right when the high to low concentration protocol was adopted. As can be seen from figure 8.8, although not significantly different, maximal responses for PGI_2 and PGE_1 for experiments using high to low protocol are noticably greater than those obtained for the low to high protocol.

8.2.1.4 Effects of drugs influencing neuronal cAMP

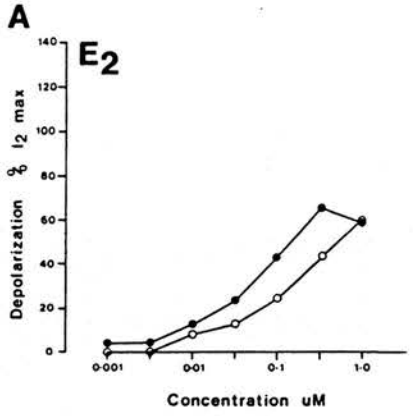
Superfusion with forskolin (1×10^{-7} - 3×10^{-5} M, n=4) or 8-Bromo cAMP (1×10^{-4} - 1×10^{-3} M, n=5) evoked concentration-dependent depolarization of the rabbit isolated vagus nerve that mimicked that produced by the active prostanoid agonists. Depolarization evoked by other compounds such as carbachol are of a much shorter duration than those produced by prostaglandins (see fig.8.9). Examples of the responses evoked by forskolin and cAMP are shown in figure 8.10. Application of the phosphodiesterase inhibitor, IBMX (1×10^{-5} - 1×10^{-4}, n=3), also caused concentration-dependent depolarization as shown in figure 8.11.

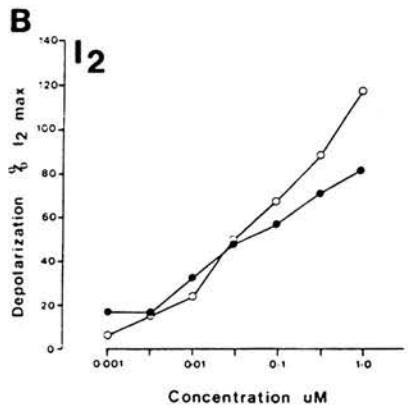
Table 8.2 Rabbit isolated vagus nerve. Summary of EC50 and Emax values for low to high (L-H) and high to low (H-L) concentration protocols.

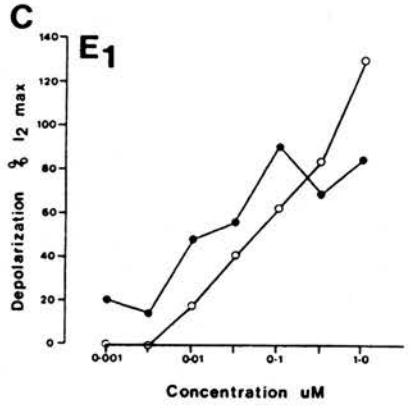
	L-H			H-L		
prostanoid agonist	EC50 (nM)	Emax (μV)	n	EC50 (nM)	Emax (μV)	n
PGE_2	79 *	489 ± 211	3	317	282 ± 41	4
PGE_1	19	513 ± 266	3	129	578 ± 230	4
PGI_2	83	600 ± 60	3	84	607 ± 205	3
cicaprost	16	413 ± 124	4	27	840 ± 219	4

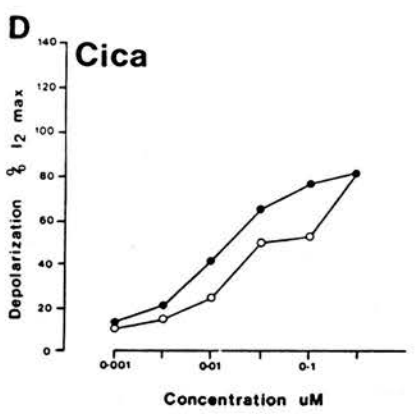
EC50 values are expressed as the geometric mean
Emax values are expressed as the arithmetic mean ± s.e.m.
* EC50 value for L-H protocol significantly different to that for H-L protocol ($p < 0.05$, Students T-test).

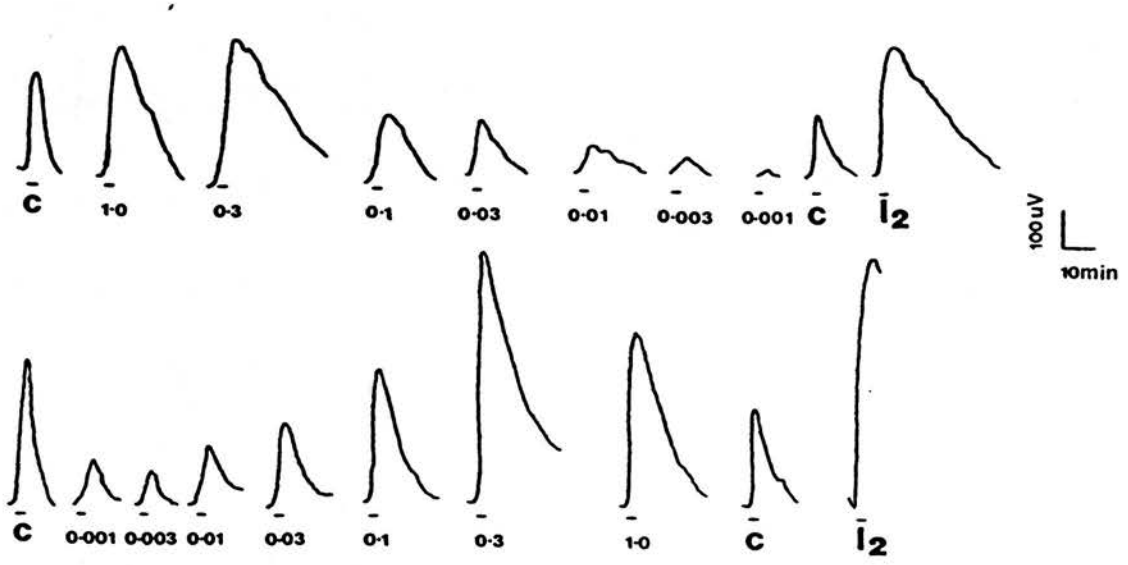
Figure 8.9 Rabbit isolalated vagus nerve. Mean log concentration-effect curves using low to high (●) or high to low (○) concentration protocols for (A) PGE_2, (B) PGI_2, (C) PGE_1 and (D) cicaprost. Mean values from n (see table 8.2) determinations are expressed as a percentage of the response evoked by a supramaximal concentration of PGI_2. Also shown in the lower panel is a discontinuous record of the effects of PGE_2 on two individual vagus nerve preparations. Both low to high and high to low protocols are shown. Standard concentrations of 0.1 mM carbachol (C), and supramaximal concentrations of 0.1 μM PGI_2 were given.


A
E_2
Depolarization % I_2 max
Concentration uM


B
I_2
Depolarization % I_2 max
Concentration uM


C
E_1
Depolarization % I_2 max
Concentration uM


D
Cica
Depolarization % I_2 max
Concentration uM


C
1·0
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0·1
0·03
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C
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0·01
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1·0
C
I_2

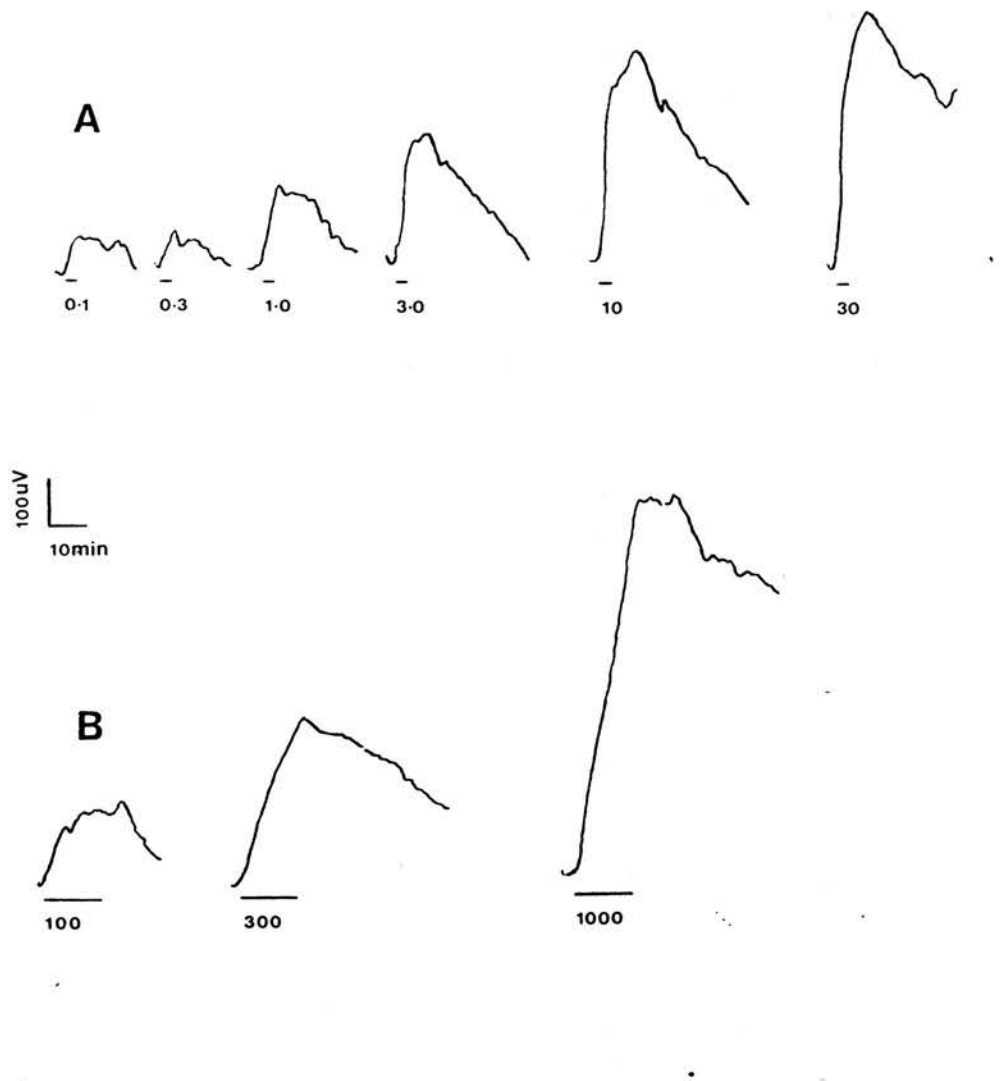

Figure 8.10 Rabbit isolated vagus nerve. Discontinuous records showing the effects of (A) forskolin and (B) cAMP on individual vagus verve preparations. Upwards deflection indicates depolarization; the solid bar under each response shows the duration of the drug application (forskolin = 3 mins, cAMP = 15 mins). Concentrations of drug applied are given in μM below each bar.

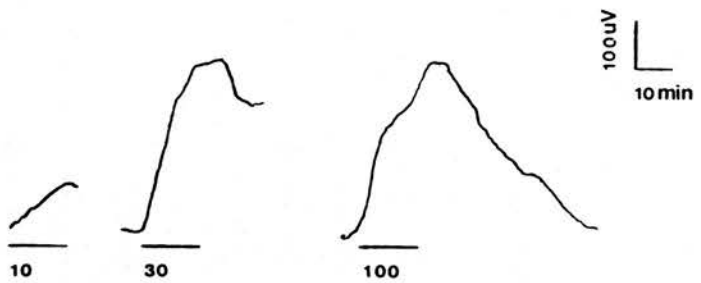

Figure 8.11 Rabbit isolated vagus nerve. Discontinuous record showing the effects of IBMX on am individual vagus nerve preparation. Upward deflection indicates depolarization; the solid bar under each response shows the duration (15 mins) of application. Concentrations of drug applied are given in μM below each bar.

8.2.2 Rat peripheral nerves

8.2.2.1 Tibial nerve

Results obtained using the tibial nerve were the most consistent of those experiments using the various peripheral nerves of the rat. PGE_2, PGE_1, PGI_2, cicaprost and iloprost evoked small, concentration-dependent depolarizations as shown in figure 8.12. At concentrations up to 10 μM $PGF_2\alpha$, PGD_2 and the TXA_2 mimetic, U46619, had no effect. Results are summarized in table 8.3.

8.2.2.2 Saphenous nerve

Once desheathed the saphenous nerve was finer in appearance than the deep tissue nerves such as the vagus, and was depolarized only weakly by PGE_2, PGI_2 and cicaprost (fig. 8.13). $PGF_2\alpha$, ICI81008, PGD_2 or U46619 had no effect at concentrations of up to 10 μM. Results are summarized in table 8.4.

8.2.2.3 Sciatic nerve

A motor branch of the sciatic nerve innervating the gastrocnemius muscle was used in these experiments. The nerve was depolarized weakly by the natural prostanoids PGE_2, PGI_2 and $PGF_2\alpha$ (fig. 8.14). One preparation responded to PGD_2. The synthetic prostanoid mimetic compounds ICI81008 and U46619 also evoked depolarization of this preparation (fig. 8.14). Results are summarized in table 8.5.

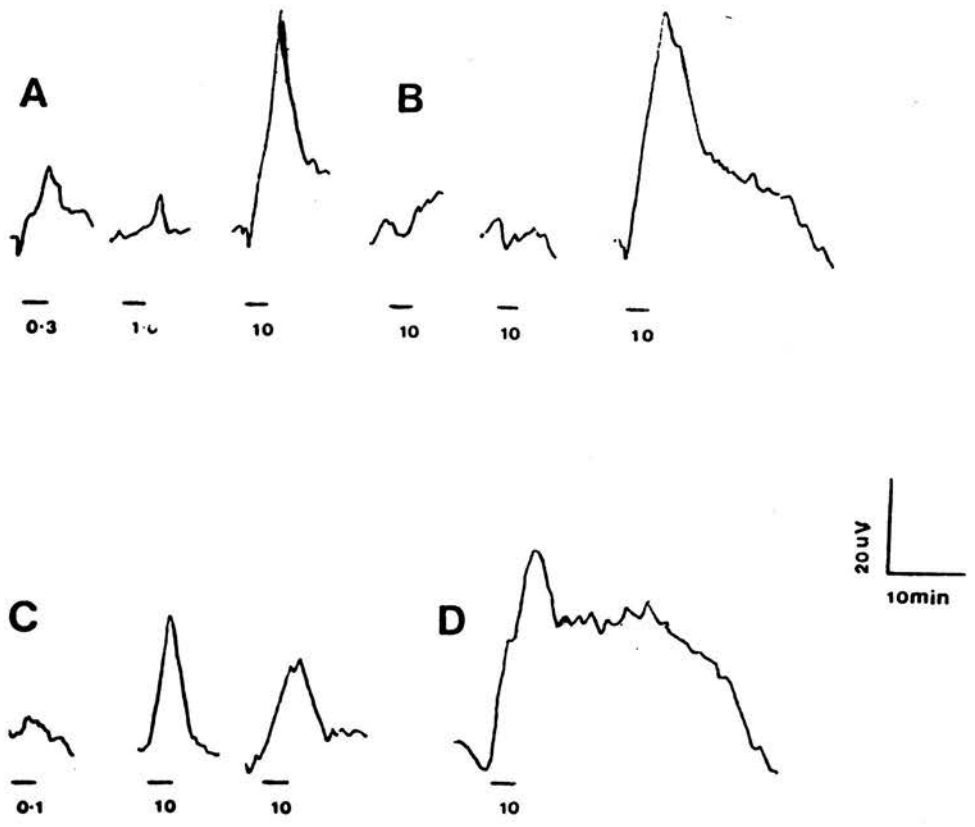

Figure 8.12 Rat isolated tibial nerve. Records illustrating the effects of (A) 0.3 - 10 uM PGI_2, (B) two applications of 10 μM PGE_2 followed by 10 μM PGI_2. On a separate tibial nerve preparation (C) shows the effect of 0.1 - 10 μM PGE_2 followed by 10 μM PGE_1, and (D) the effect of 10 μM cicaprost. Upwards deflection indicates depolarization; the solid bar under each response shows the duration of application (3 mins). Concentrations of drug applied are given in μM below each bar.

Table 8.3 Rat tibial nerve. Summary of prostanoid-induced depolarization. PGE_2, PGE_1, cicaprost and iloprost gave he most consistent responses following application at concentrations of up to 10μM.

prostanoid agonist	responses/applications	% responding tissues
PGE_2	5/8	62.5
PGE_1	1/2	50
cicaprost	3/3	100
iloprost	1/1	100
$PGF_{2\alpha}$	0/2	0
PGD_2	0/2	0
U46619	0/1	0

The figures given were taken from experiments on a total of ten preparations.

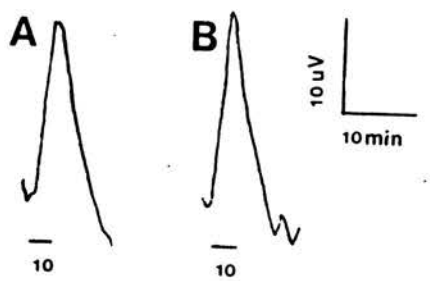

Figure 8.13 Rat isolated saphenous nerve. Records illustrating the effects of 10 μM (A) cicaprost and (B) PGE_2 on an idividual saphenous nerve preparation. Upward deflections indicate depolarization; the solid bar under each response indicates the duration of application (3 mins). Concentrations of drug applied are given in μM below each bar.

Table 8.4 Rat isolated saphenous nerve. Summary of prostanoid-induced depolarizations. PGE_2, PGE_1 and PGI_2 gave the most consistent responses following application at concentrations of 10μM.

prostanoid agonist	responses/applications	% responding tissues
PGE_2	3/8	37.5
PGI_2	2/2	100
cicaprost	3/8	37.5
$PGF_{2\alpha}$	0/4	0
PGD_2	1/7	14.3
U46619	0/1	0

The figures given were taken from experiments on a total of four preparations.

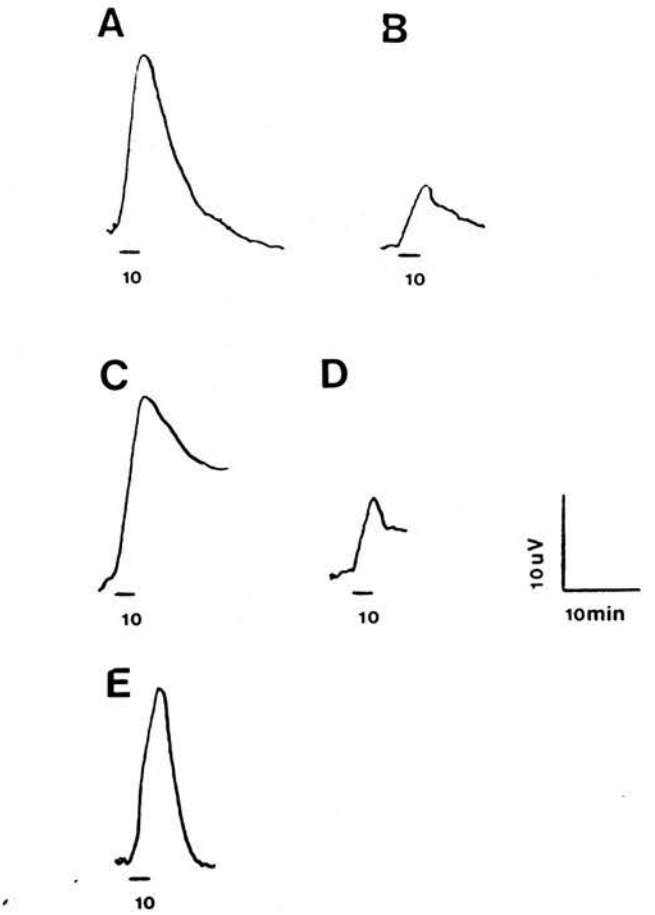

Figure 8.14 Rat isolated sciatic nerve. Record illustrating the effect of (A) 10 μM $PGF_2\alpha$ follwed by (B) 10μM U46619 on an individual sciatic nerve. On a separate preparation (C) shows the effect of 10μM $PGF_2\alpha$ followed by (D) 10μM PGI_2. (E) Illustrates the effect of 10 μM PGI_2 on an individual preparation. Upwards deflections indicate depolarization; the solid bar under each response shows the duration of application (3 mins). Concentrations of drug applied are given in μM below each bar.

Table 8.5 Rat isolated sciatic nerve. Summary of prostanoid-induced depolarizations. $PGF_{2\alpha}$ and ICI81008 gave the most consistent responses, with PGE_2, PGE_1, PGI_2 and U46619 producing relatively consistent depolarizations following application of concentrations up to 10μM.

prostanoid agonist	responses/applications	% responding tissues
PGE_2	3/7	43
PGI_2	3/6	50
$PGF_{2\alpha}$	6/6	100
ICI81008	2/2	100
PGD_2	1/5	20
U46619	2/3	67

The figures given were taken from experiments on a total of three preparations.

8.3 DISCUSSION

8.3.1 Rabbit vagus nerve

The potent depolarizing effects of PGE_2, PGE_1, PGI_2 and the selective IP-receptor agonists, cicaprost and iloprost, suggest that populations of both EP- and IP-receptors may be present on axons of the rabbit vagus nerve. The relatively high potency of PGE_2 on the rabbit vagus compared with that of PGE_1 and PGI_2 is in contrast to that seen for the rat vagus where PGE_1 and PGI_2 are approximately 100 fold more potent than PGE_2 (Poll et al., personal communication). Species differences in EP-receptor populations between the rat and the rabbit may explain this differential relative potency on the two preparations.

Results from experiments on rabbit isolated vagus using selective agonists for EP_1-, EP_2- and EP_3-receptors suggest that the receptor mediating the depolarizing effect of PGE_2 does not belong to any of the recognised receptor subtypes. The relatively shallow slope of the PGE_2 log concentration-effect curve suggests that a selective desensitization of the preparation to the action of PGE_2 may occur. Desensitization to the depolarizing effects of PGE_2 is supported by the finding that using a high to low concentration protocol caused a significant shift of the log concentration-effect curve to the right. Other than desensitization, these effects may have been produced as a result of an action of higher concentrations of PGE_2 at a receptor subtype mediating inhibitory effects. Inhibitory effects of high doses of PGE_2 on nociceptive sensory receptors has also been described in the present series of investigations (see Section VII).

The relatively high potency of PGE_1, compared to that of PGE_2, on vagal preparations of both the rabbit and rat suggests that PGE_1 is acting at IP-receptors as well as EP-receptors. Desensitization to PGE_1 was not significant in the rabbit vagus, providing evidence of an action predominantly at IP-receptors. This is particularly clear in the rat vagus where PGE_1 and PGI_2 are equipotent, while PGE_2 is a 100 fold weaker (Poll et al., personal communication). It should also be noted that in the desensitization experiments using high to low concentration protocols the mean maximal response for PGI_2 and PGE_1 were noticeably greater than those for low to high protocols. This may be explained in terms of a desensitization of responses to PGI_2 and PGE_1 . Thus, when low to high concentration protocols are adopted with PGI_2 or PGE_1 as the agonist, a slow desensitization of either an IP- or EP-receptor will occur, resulting in a smaller maximum response and a shallower slope of the log concentration-effect curve. Further studies using selective desensitization or selective antagonists may help to clarify whether EP-, IP- or both receptor types are involved.

The vagus nerve of the rabbit has been shown to be largely composed of C fibres (Evans & Murray, 1954), a fibre type that is considered to be involved in afferent transmission of nociceptive stimuli (see Besson & Chaouch, 1987). It has recently been demonstrated that transport of receptors for various endogenous substances from cell bodies to peripheral nerve terminals occurs in the vagus nerve (Young et al., 1980). Assuming that the prostanoid receptors expressed on axons of the vagus nerve are the same as those present at sensory terminals, the present results are in agreement with the existing literature describing PGE_1 or PGI_2 as the most potent naturally occurring prostanoids acting

to increase the excitability of nociceptive sensory receptors (Bergstrom et al., 1959; Gillespie, 1972; Collier & Schneider, 1972; Willis & Cornelson, 1973; Ferreira et al., 1973; James & Church, 1978; Ferreira et al., 1978; Tyers & Haywood, 1979; Juan, 1979). Of particular interest is the finding that prostanoids of the E-series increase the spontaneous firing rate of single vagal pulmonary and bronchial afferent C fibres when injected into the right atrium or nebulized into the lungs of anaesthetized dogs (Coleridge et al., 1976). Since in the present study, PGE_1 and PGI_2 were more potent than PGE_2, and as arachadonic acid is the major mammalian precursor to prostanoid synthesis, PGI_2 and not PGE_1 is the more likely candidate for the role of endogenous mediator. However, it has been demonstrated indirectly that PGI_2 is converted to 6-keto-PGE_1 in the platelet (Wong et al., 1980; Griffiths and Moore, 1983), in the lung (Berry et al., 1986), and the kidney (Griffiths and Moore, 1983). Conversion to 6-keto-PGE_1 has now also been measured directly for the kidney (Pieroni et al., 1988). 6-keto-PGE_1 shares many actions with PGI_2, including vasodilatation (Quilley et al., 1979), inhibition of platelet aggregation (Wong et al., 1979; Quilley et al., 1980), and promotion of renin secretion (Jackson et al., 1981). It is possible that conversion of PGI_2 to 6-keto-PGE_1 may provide a mechanism for extending the actions of PGI_2, where the conversion product is a highly potent IP-receptor agonist. There are obvious implications concerning prostanoid-induced hyperalgesia, and as such it would be of great interest to examine the effects of 6-keto-PGE_1 on sensory nerves.

In contrast to the depolarizing effect of PGD_2 on the rabbit nodose ganglion neurones (Fowler et al., 1987), This prostanoid was innefective on the vagus nerves of the rabbit or the rat (Poll et al., personal

communication). Differential expression and/or transport of receptors between the cell bodies and peripheral or central axon terminals of sensory neurones may explain these findings.

The ability of forskolin and 8-bromo cAMP to mimic the depolarizing effects of the natural prostanoid agonists in the rabbit isolated vagus, raises the possibility that the prostanoids are acting via cAMP in this preparation. This is supported by the finding that PGE_1 causes a marked accumulation of cAMP when incubated with sections of desheathed rabbit vagus nerve (Kalix, 1979). In addition, neuroblastoma cells in culture react to the presence of low concentrations of PGE_1 with increases in their cAMP content (Gilman et al., 1971). In the present study the depolarizing action of the phosphodiesterase inhibitor IBMX on the rabbit vagus indicates that there is a basal turnover of cAMP in the rabbit isolated vagus. Inhibition of the phosphodiesterase enzyme by IBMX, unmasks this basal production of cAMP resulting in nerve depolarization.

Prostanoid-induced hyperalgesia has been postulated by Ferreira & Nakamura (1979) to be mediated via cAMP. They demonstrated that hyperalgesia developed following subplantar injection of PGE_2, PGI_2 or dibuteryl cAMP into rat paws. The sensitizing effects of these agents were enhanced by the presence of phosphodiesterase inhibitors, which inhibit the metabolism of cAMP. Clues to the mechanism by which prostanoids may cause sensitization of sensory receptors via cAMP are provided by results from studies on rabbit C-type nodose ganglion neurones <u>in vitro</u>, where PGE_2, PGE_1 and PGD_2 cause the inhibition of a slow after-hyperpolarization via the blockade of a Ca^{2+}-dependent K^+ channel (Fowler et al., 1985a; 1985b; Weinreich & Wonderlin, 1987). This

effect is mimicked by forskolin, suggesting that inhibition is mediated via cAMP (Weinreich & Wonderlin, 1987). Prostaglandins also increase the excitability of dorsal root ganglion neurones in culture (Baccaglini & Hogan, 1983), although the mechanism involved has not yet been identified.

8.3.2 Rat peripheral nerves

Isolated sensory nerve and mixed nerve preparations used in this study were weakly depolarized by PGE_2, PGE_1, PGI_2 and its stable analogues iloprost and cicaprost. $PGF_{2\alpha}$, PGD_2 or U46619 had no effect on the resting potential of these nerves. In the isolated motor nerve, all of these prostanoids and their stable analogues evoked weak depolarizations, with $PGF_{2\alpha}$ producing the most consistent response.

The small size and inconsistent nature of the responses obtained in these experiments may be a result of the small number of fibres responding to applied drugs, and the inability of the recording equipment used to pick up the resulting depolarizations. Although the construction of concentration effect curves was not possible, a rough guide as to which prostanoids may effect the nerves under study has been produced. Results obtained with the saphenous and tibial nerves are in agreement with those from the rat isolated vagus (Poll et al., personal communication), lending further support to the suggestion that IP- and perhaps EP-receptor populations are present on peripheral sensory nerves of the rat. The additional presence of FP- and perhaps TP-receptors on motor nerves is indicated by the depolarizing actions of $PGF_{2\alpha}$, the selective FP-receptor agonist ICI81008, and the stable TXA_2 mimetic

U46619 on the motor branch of the sciatic nerve. Evidence exists that prostanoids can affect the functions of motor nerves in other systems. There are reports suggesting that prostaglandin-like substances increase the release of acetylcholine from guinea pig ileum (Bergami et al., 1978). Prostanoids of the E-series, PGI_2 and to a lesser extent $PGF_{2\alpha}$, augment the cholinergically-mediated contractions obtained in response to electrical field stimulation of the guinea pig ileum longitudinal muscle-myenteric plexus preparation (Poll et al., 1988). Indomethacin and phenylbutazone have been reported to increase the frequency of miniature endplate potentials in frog sartorius muscle (Madden and Kloot, 1980). Finally, a direct effect of PGE_1, to alter amplitude and conduction velocity of the compound action potential in frog sciatic nerve has been demonstrated by Horrobin et al. (1977).

8.4 CONCLUSIONS

Both in the rabbit and the rat, prostanoid-induced depolarization of peripheral sensory nerve axons appear to be mediated by populations of IP- and perhaps EP-receptors. In addition to these receptor types, populations of FP and TP-receptors mediating depolarization may also be associated with motor nerves. The depolarizing actions of the prostanoids may be mediated via stimulation of intracellular cAMP production. These findings support those of previous studies showing that PGI_2 and PGE_2 act as potent hyperalgesic agents, and produce direct excitation of nociceptive sensory receptors (see Section VII).

SECTION IX

COMPARISON OF THE EFFECTS OF LYSINE-ACELTYLSALICYLATE, SODIUM SALICYLATE AND PARACETAMOL ON AFFERENT DISCHARGE FROM ARTICULAR MECHANONOCICEPTORS FROM ARTHRITIC ANKLE JOINTS IN THE RAT

SECTION IX

COMPARISON OF THE EFFECTS OF LYSINE-ACELTYLSALICYLATE, SODIUM SALICYLATE AND PARACETAMOL ON AFFERENT DISCHARGE FROM ARTICULAR MECHANONOCICEPTORS FROM ARTHRITIC ANKLE JOINTS IN THE RAT

9.1 INTRODUCTION

A peripheral site of analgesic action for non-steroidal anti-inflammatory drugs (NSAIDS) was originally demonstrated by Lim and colleagues (Guzman et al., 1964; Lim, 1970) in the dog spleen model of bradykinin-induced pain. The concept of a peripheral action of analgesic agents was supported by the observations that certain prostaglandins potentiate nociceptive responses to mechanical or chemical stimuli (Ferreira, 1972; Rosenthale et al., 1972; Ferreira et al., 1973; Willis & Cornelsen, 1973; Lembeck & Juan, 1974; Moncada et al., 1975; Chahl & Iggo, 1977), and that NSAIDS inhibit the formation of prostanoids in different tissues (Ferreira et al., 1971; Vane, 1971; Ferreira & Vane, 1974; Moncada et al., 1975; Roth et al., 1975; Ferreira et al, 1978; Higgs & Salmon, 1979).

More recently it has been shown that aspirin reduces the excitation of nociceptive sensory afferents evoked by bradykinin in cat muscle (Mense, 1982). Furthermore, the sensitization of articular nociceptive afferents induced by either chronic arthritis in the rat (Guilbaud & Iggo, 1985)

or acute joint inflammation in the cat (Heppelmann et al., 1986), has been demonstrated to be depressed by the administration of aspirin. These effects on inflamed joints can be at least partially overcome by the subsequent administration of exogenous prostanoids (Heppelmann et al., 1986; see Section VII), suggesting that a reduction in endogenous prostanoids in the periphery is responsible for the reduction in nociceptor sensitivity caused by aspirin.

Arguments against a purely peripheral action of NSAIDS have, however, been made (see Jurna & Brune, 1990), and the actions of agents such as paracetamol, which display antipyretic and analgesic effects without significant anti-inflammatory actions, are often considered to have an exclusively central action (see Goodman & Gilman, 1985; Rang & Dale, 1987). Furthermore, paracetamol only weakly inhibits the formation of prostaglandins in peripheral tissue (Flower et al., 1972; Brune et al., 1981), but potently reduces cyclooxygenase activity in brain tissue (Flower et al., 1972; Flower & Vane, 1972).

In the present series of experiments the effects of paracetamol were compared with those of lysine acetylsalicylate (l-AS) on the discharge characteristics of articular mechanonociceptors from arthritic ankle joints. These experiments were carried out to provide information regarding whether or not paracetamol has peripheral actions comparable with those of aspirin. In addition, sodium salicylate was used in order to determine whether the acetyl group of l-AS is required for activity. In a separate study the effects of l-AS, sodium salicylate or paracetamol were examined on tissue levels of PGE_2 and 6-keto $PGF_{1\alpha}$, the stable metabolite of PGI_2, in chronically inflamed capsular tissue from the rat ankle joint.

9.2 RESULTS

The effects of 1-AS, sodium salicylate and paracetamol were examined in nineteen experiments, from which twenty three high-threshold slowly adapting mechanoreceptors were identified and studied. Mechanonociceptors from arthritic ankle joints had afferent fibre conduction velocities in the range 0.25 - 2 ms^{-1} (mean: 0.8 ± 0.1 ms^{-1}), indicating that these were C fibres. All of the twenty three units studied had an irregular resting discharge (mean: 1.4 ± 0.4 i.p.s), before the injection of any drugs.

9.2.1 Mechanonociceptor responsiveness

Administration of 1-AS (100 $mgkg^{-1}$, equivalent to 50 $mgkg^{-1}$ ASA, i.v.), caused a significant reduction in the discharge evoked by the standard mechanical stimulus in all eleven units examined (fig. 9.1). The mean latency to the onset of the effect was 9.8 ± 2.4 minutes (range: 2 - 28 mins). Mechanoreceptor responsiveness reached a mean minimal value of 51% (range: 0 - 73%) of the pre-injection control following periods ranging from 8 - 32 minutes for individual units (mean: 20 ± 2.7 mins).

Sodium salicylate (50 $mgkg^{-1}$, i.v.) caused a reduction in the response to mechanical stimuli in two of three units (fig. 9.2). In these two units the effect had latencies to onset of 4 and 16 minutes. Minimum mechanonociceptor responsiveness was reached after 34 and 32 minutes, and had values of 6 and 50% of pre-injection control respectively.

Injection of paracetamol (50 $mgkg^{-1}$, i.v.), significantly reduced mechanoreceptor responsiveness to mechanical stimuli in seven (100%) of

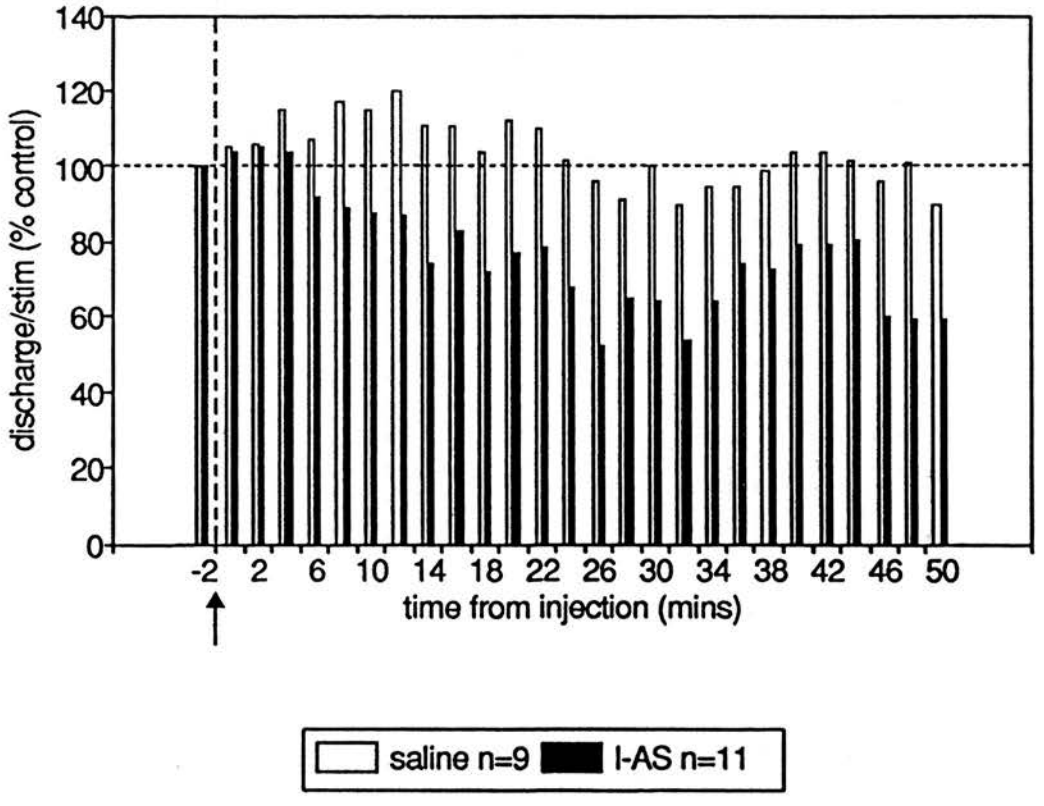

Figure 9.1 Summary of the effects of l-AS on mechanonociceptor responsiveness to mechanical stimuli. Each bar represents the mean response of n units to a standard mechanical stimulus, applied once every two minutes, both before and after the i.v. injection of l-AS (50 $mgkg^{-1}$, ASA equivalent) or saline vehicle. Values are expressed as a percentage of the pre-injection control reponse to the mechanical stimulus. The arrow and vertical dashed line indicate the time of injection, the first mechanical stimulus being delivered 15s after this time.

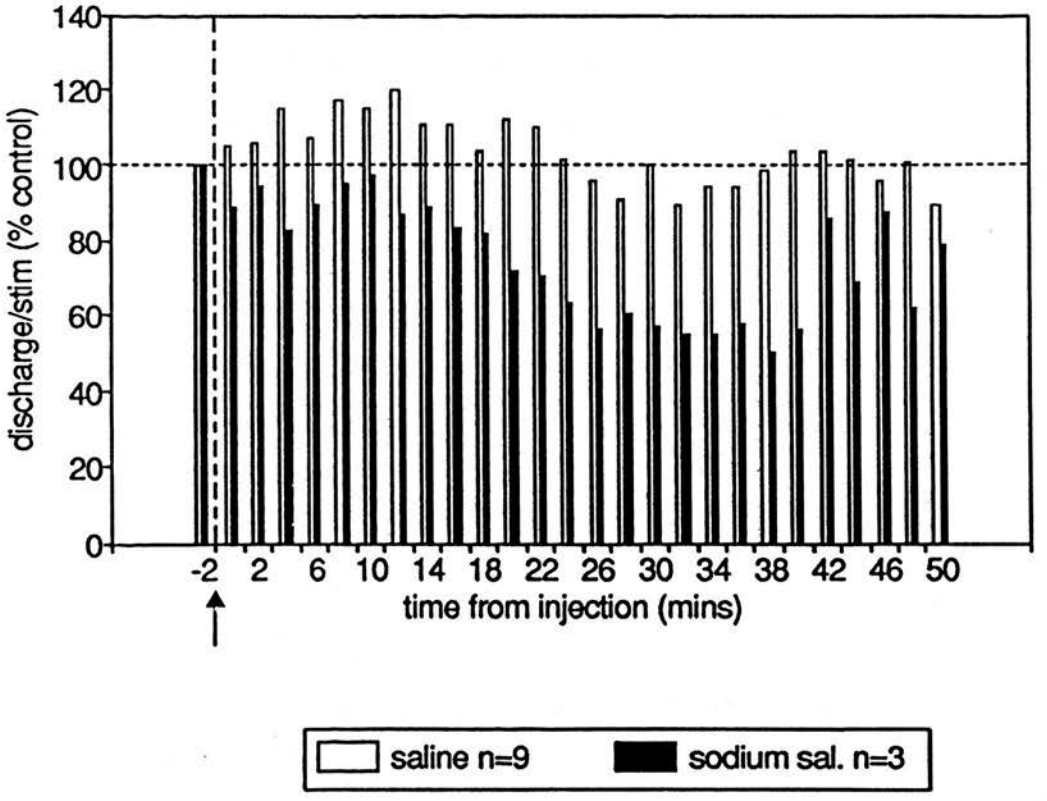

Figure 9.2 Summary of the effects of sodium salicylate on mechanonociceptor responsiveness to mechanical stimuli. Each bar represents the mean response of n units to a standard mechanical stimulus, applied once every two minutes, both before and after the i.v. injection of sodium salicylate (50 $mgkg^{-1}$) or saline vehicle. Values are expressed as a percentage of the pre-injection control reponse to the mechanical stimulus. The vertical dashed line indicates the point of injection, the first mechanical stimulus being delivered 15s after this time.

seven units examined (fig. 9.3). The mean latency to the onset of the effect was 6.6 ± 1.9 minutes (range: 4 - 18 minutes). Mechanonociceptor responsiveness reached a mean minimal value of 51% (range: 0 - 70%) of the pre-injection control, following a delay ranging from 4 - 20 minutes (mean: 15 ± 2 mins).

A summary of the effects of 1-AS, sodium salicylate and paracetamol is shown in figure 9.4, and illustrates that the three drugs cause reductions in mechanoreceptor responsiveness of similar time course and amplitude. All caused statistically significant reductions when compared with changes in mechanoreceptor responsiveness following saline injection. Although in the majority of experiments prostanoids were injected 30 - 60 minutes after the administration of 1-AS, sodium salicylate or paracetamol, following depression of mechanoreceptor responsiveness, recovery to pre-injection levels of responsiveness was not seen for recording periods of up to 70 minutes.

9.2.2 Spontaneous mechanonociceptor discharge

Injection of 1-AS (50 $mgkg^{-1}$, ASA equivalent, i.v.), caused a significant reduction in ongoing discharge in all eleven units (100%) examined (fig. 9.5). The reduction in discharge had a mean latency to onset of 4.6 ± 1.3 minutes (range: 2 - 15 mins). Ongoing discharge reached a mean minimum value of 39% of pre-injection control (range: 0 - 73%), after a delay in the range 3 - 25 minutes (mean: 10 ± 1.8 mins).

Sodium salicylate (50 $mgkg^{-1}$, i.v.), reduced ongoing discharge in three of three units examined (fig. 9.6). This reduction had a mean latency to onset of 5.3 ± 2 minutes (range: 2 - 9 mins), and the minimum

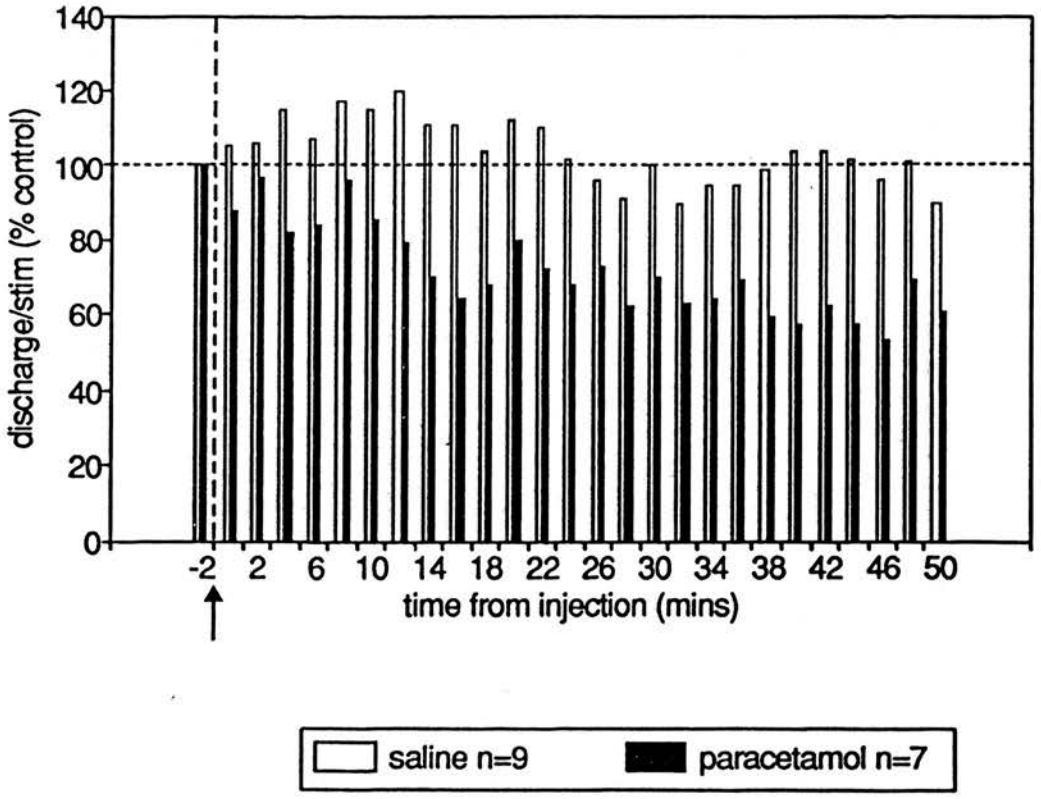

Figure 9.3 Summary of the effects of paracetamol on mechanonociceptor responsiveness to mechanical stimuli. Each bar represents the mean response of n units to a standard mechanical stimulus, applied once every two minutes, both before and after the i.v. injection of paracetamol (50 $mgkg^{-1}$) or saline vehicle. Values are expressed as a percentage of the pre-injection control reponse to the mechanical stimulus. The vertical dashed line indicates the point of injection, the first mechanical stimulus being delivered 15s after this time.

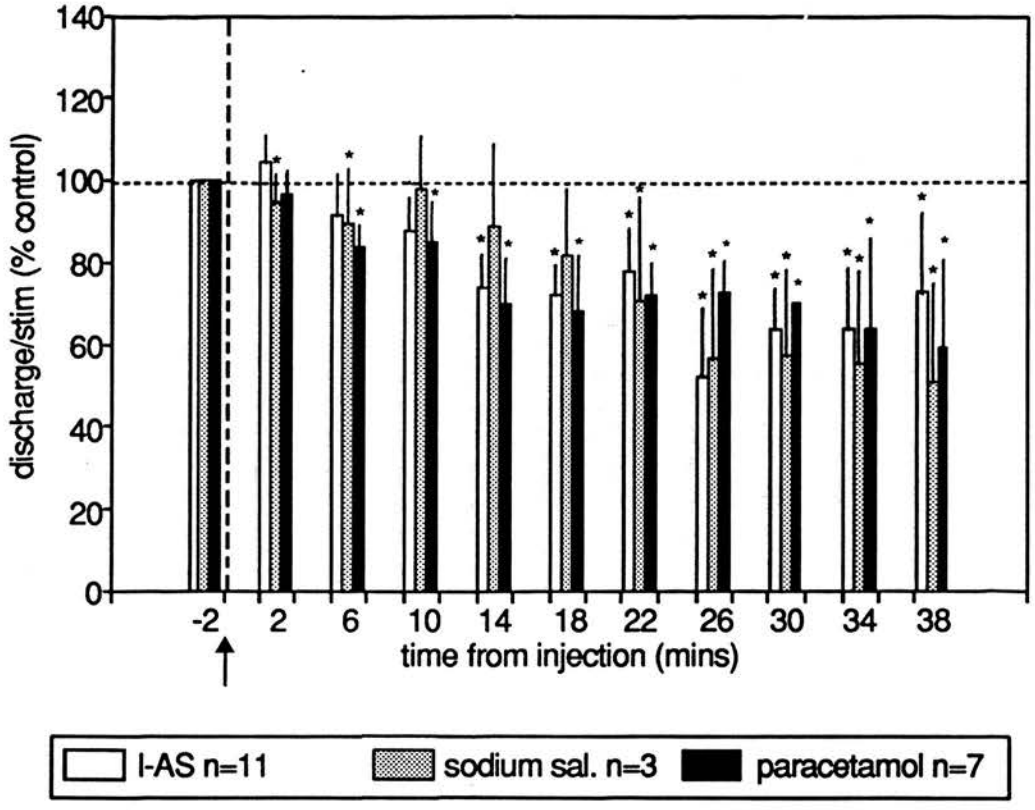

Figure 9.4 Comparison of the effects of l-AS, sodium salicylate and paracetamol on mechanonociceptor responsiveness to mechanical stimuli. Each bar represents the mean response of n units to mechanical stimuli both before and after the i.v. injection of l-AS (50mgkg^{-1}, ASA equivalent), sodium salicylate (50mgkg^{-1}) or paracetamol (50 mgkg^{-1}). The arrow and vertical dashed line indicate the time point of injection. Values are expressed as a percentage of the pre-injection control response, and the vertical lines above each bar represent the s.e.m. These mean values illustrate that the depressant effects of all three drugs follow a similar time course. The rate of decline in mechanoreceptor responsiveness varied between units, this being reflected in the size of the error bars. Values significantly ($p < 0.05$) different from those obtained following injection of saline (shown in figures 10.1 - 10.3) are indicated as * (Wilcoxon).

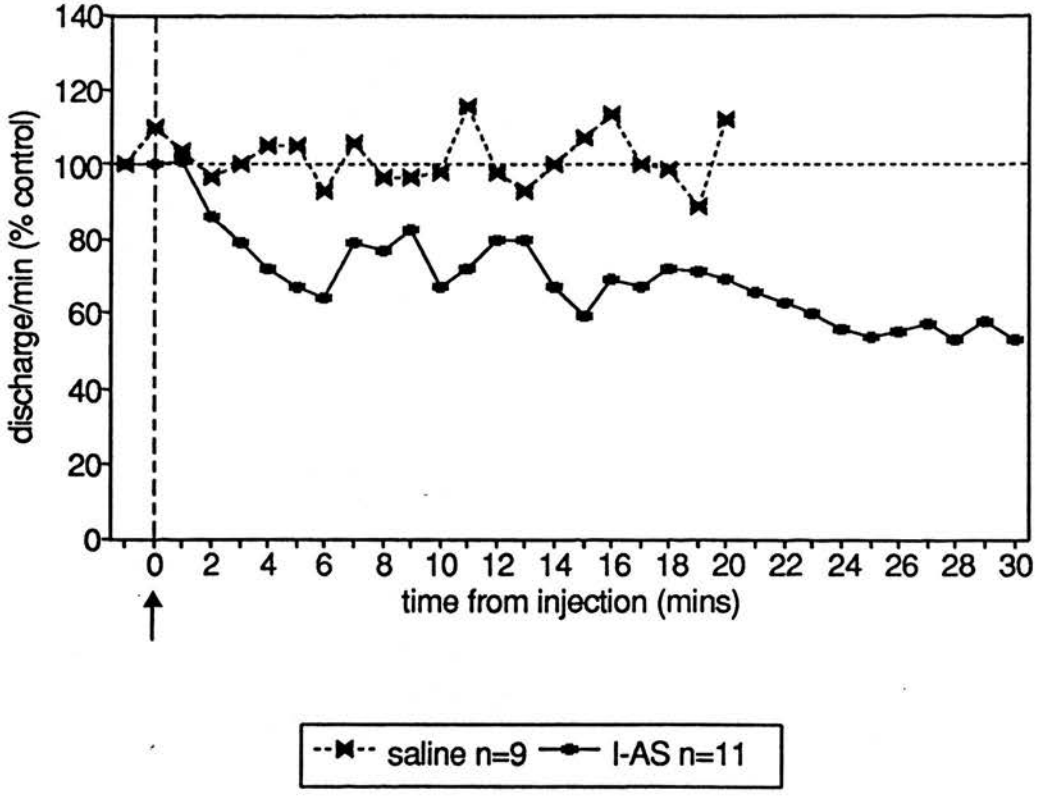

Figure 9.5 Summary of the effects of l-AS on spontaneous mechanonociceptor discharge. Each point represents the mean discharge of n units, measured over a 1 min period, both before and after the i.v. injection of l-AS (50 $mgkg^{-1}$, ASA equivalent) or saline vehicle. Values are expressed as a percentage of the pre-injection control discharge (1.7 ± 0.6 i.p.s.). The arrow and vertical dashed line indicate the time of injection.

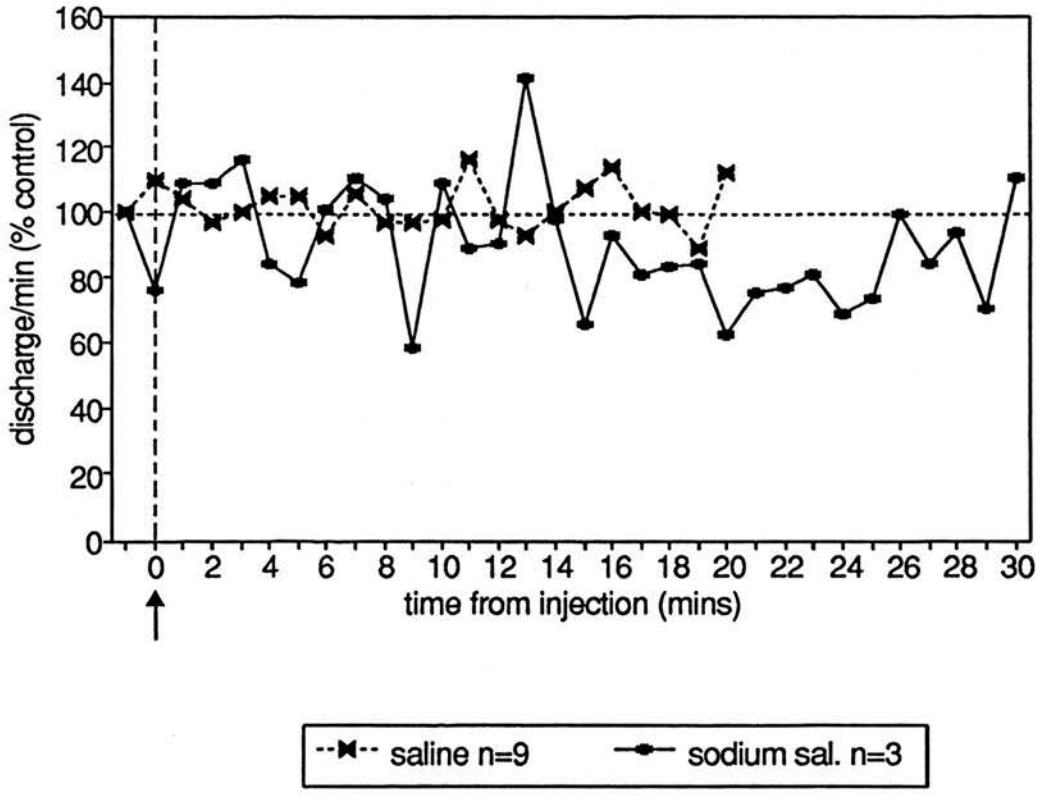

Figure 9.6 Summary of the effects of sodium salicylate on spontaneous mechanonociceptor discharge. Each point represents the mean discharge of n units, measured over a 1 min period, both before and after the i.v. injection of sodium salicylate (50 mgkg^{-1}, ASA equivalent) or saline vehicle. Values are expressed as a percentage of the pre-injection control discharge (0.4 ± 0.1). The arrow and vertical dashed line indicate the time point of injection. A large variation in levels of discharge following sodium salicylate injection was seen.

discharge averaged 29% of the pre-injection control after periods ranging from 9 - 23 minutes (mean: 14 ± 4.4 mins).

Injection of paracetamol (50 $mgkg^{-1}$, i.v.) caused a significant reduction in ongoing discharge in nine (100%) of nine units examined (fig. 9.7). A mean delay of 5 ± 0.8 minutes (range 2 - 10 minutes) was seen before the onset of the reduction in ongoing discharge. A mean minimum level of discharge equal to 50% of pre-injection control (range 0 - 78%) was reached following periods ranging from 8 - 20 minutes (mean: 12.6 ± 1.7 mins).

Figure 9.8 summarizes the effects of 1-AS, sodium salicylate and paracetamol on the ongoing discharge. All drugs had a similar time course and amplitude of effect, producing statistically significant reductions in afferent discharge when compared with the effect of saline injection. Although in the majority of experiments prostanoids were injected 30 - 60 minutes after the administration of 1-AS, sodium salicylate or paracetamol recovery to pre-injection levels of discharge was not seen for observation periods of up to 70 minutes.

9.2.3 Additive effects of paracetamol and 1-AS

Injection of a second dose of 1-AS (50 $mgkg^{-1}$, ASA equivalent, i.v.) in three experiments, or paracetamol (50 $mgkg^{-1}$, i.v.) in another three experiments, did not cause any further reduction in either ongoing activity or mechanonociceptor responsiveness when administered 40 - 60 minutes after the initial dose. In three experiments administration of 1-AS (50 $mgkg^{-1}$, ASA equivalent, i.v.) 30 - 50 minutes after an initial injection of paracetamol (50 $mgkg^{-1}$, i.v.), caused a further, although

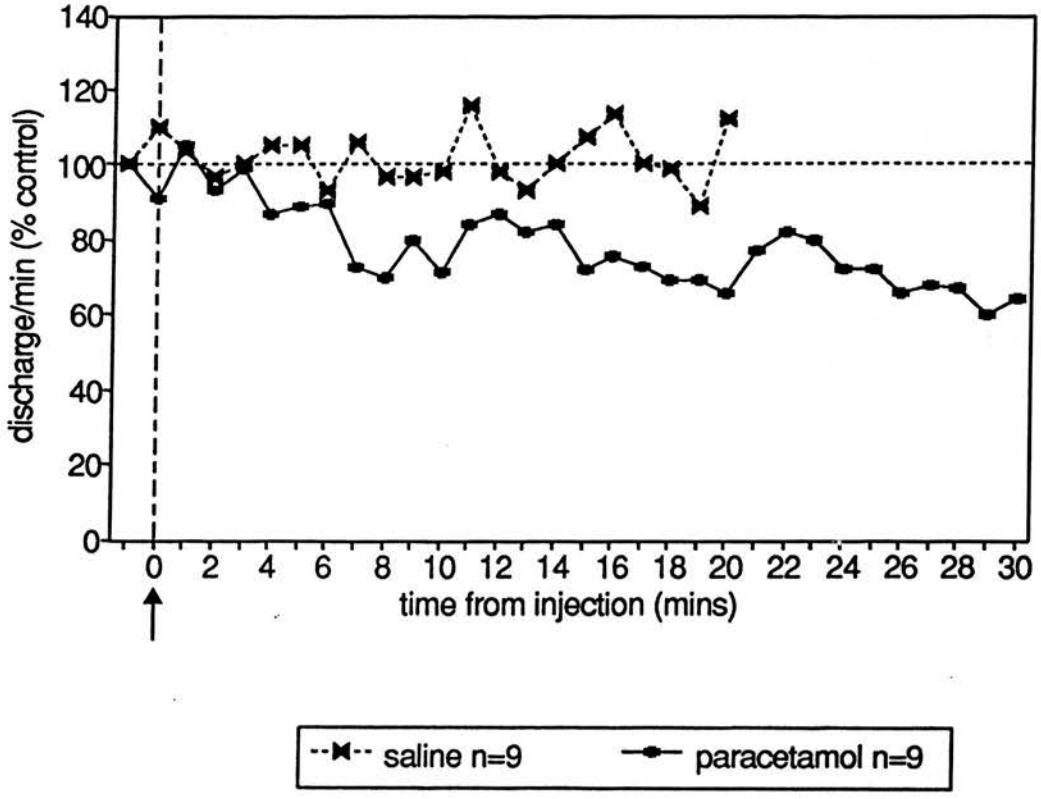

Figure 9.7 Summary of the effects of paracetamol on spontaneous mechanonociceptor discharge. Each point represents the mean discharge of n units, measured over a 1 min period, both before and after the i.v. injection of paracetamol (50 $mgkg^{-1}$) or saline vehicle. Values are expressed as a percentage of the pre-injection control discharge (1.2 ± 0.4 i.p.s.). The arrow and vertical dashed line indicate the time point of injection.

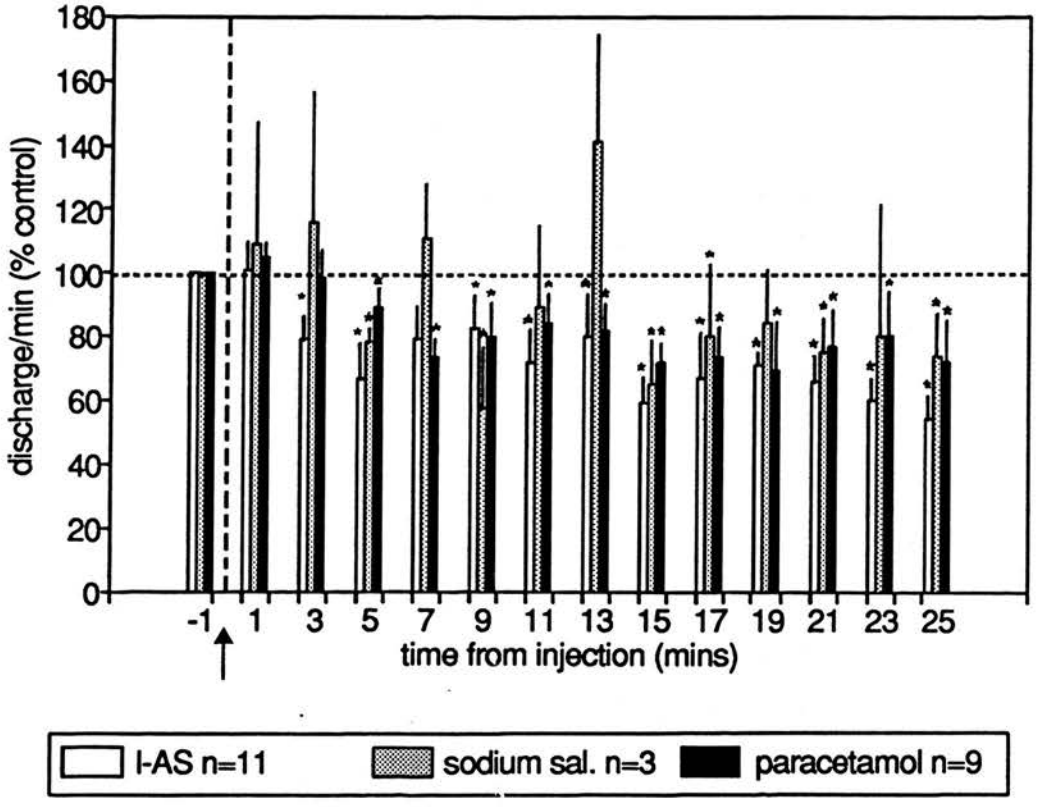

Figure 9.8 Comparison of the effects of l-AS, sodium salicylate and paracetamol on spontaneous mechanonociceptor discharge. Each bar represents the mean discharge of n units before and after the i.v. injection of l-AS (50mgkg^{-1} (50 mgkg^{-1}). The arrow and vertical dashed line indicate the time point of injection. Values are expressed as a percentage of the pre-injection control discharge, and the vertical lines above each bar represent the s.e.m. These mean values illustrate that the depressant effects of all three drugs follow a similar time course. The depressant effect of sodium salicylate was less consistent and more variable, this being reflected in the size of the error bars. Values significantly ($p < 0.05$) different those obtained following injection of saline are shown as * (Wilcoxon).

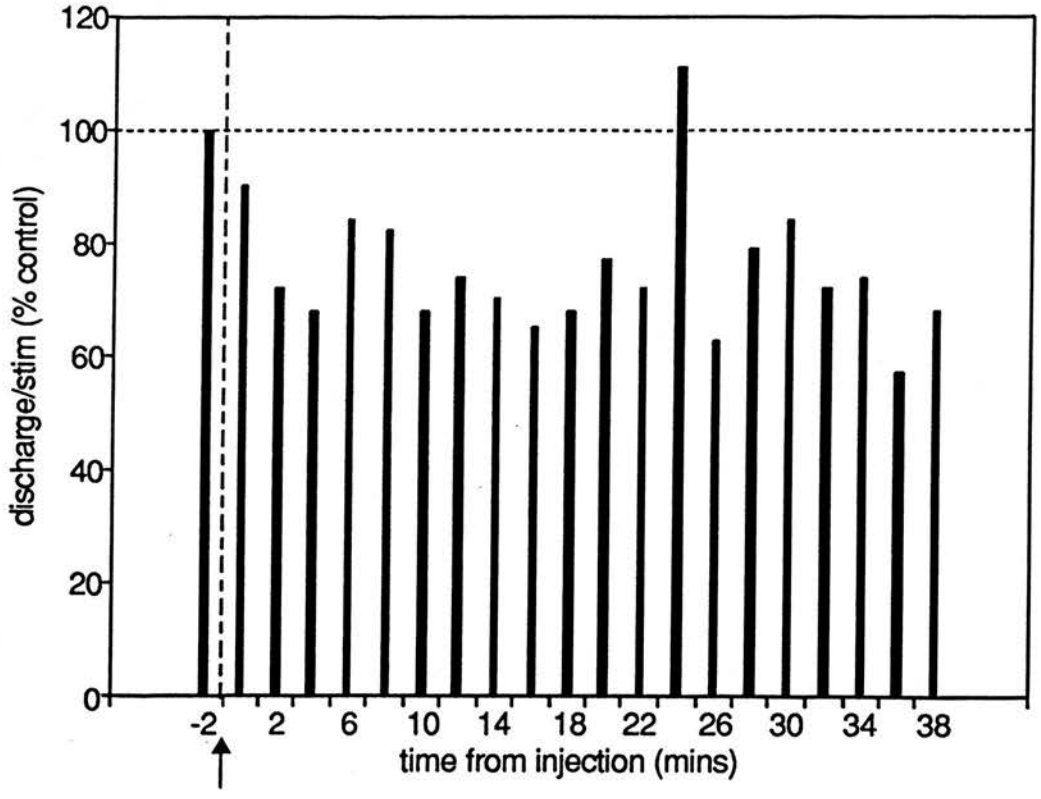

Figure 9.9 Effect of l-AS after paracetamol on mechanonociceptor responsiveness to mechanical stimuli. Each bar represents the response of a single unit to a standard mechanical stimulus applied once every two minutes, both before and after the injection of l-AS (50 $mgkg^{-1}$, ASA equivalent, i.v.). The arrow and vertical dashed line indicate the time point of injection. Values are given as a percentage of the pre-injection control response.

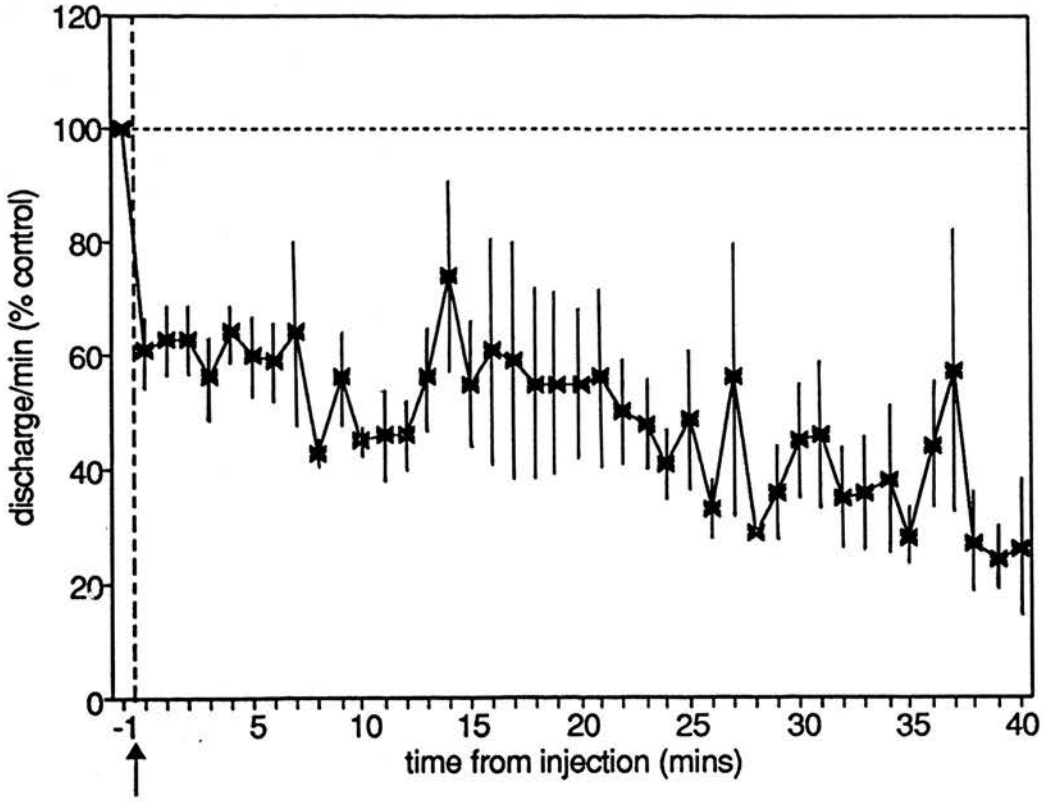

Figure 9.10 Effects of 1-AS on spontaneous mechanonociceptor discharge after paracetamol. Each point represents the mean discharge of 3 units both before and after the injection of 1-AS (50 $mgkg^{-1}$ ASA equivalent, i.v.). Values are given as a percentage of the pre-injection control discharge, and the vertical bars represent ± s.e.m.. The arrow and vertical dashed line indicate the time at which 1-AS was injected. A rapid onset reduction in discharge is clearly evident.

not statistically significant, reduction both in mechanical responsiveness and in ongoing discharge (figs. 9.9 - 9.10).

9.2.4 Effects of cicaprost on paracetamol-induced depression of mechanoreceptor discharge

As described in Section VII of this thesis, following depression by 1-AS, injection of cicaprost (0.01 - 1 μg, i.a.), caused an increase both in mechanonociceptor responsiveness, and in ongoing activity. When administered 30 - 60 minutes after the injection of paracetamol (50 $mgkg^{-1}$, i.v.), injection of cicaprost (0.01 - 1 μg, i.a.) caused similar increases in mechanonociceptor discharge two units. These responses were dose-dependent, had a short latency to onset of approximately 15 seconds, and had a long duration of over 20 minutes. A summary of the effects of cicaprost on mechanonociceptor responsiveness is given in figure 9.11, and an example of its effect on ongoing activity is shown in figure 9.12. In a separate experiment injection of cicaprost (0.01 -1 μg, i.a.) also partially reversed the depressant effect of sodium salicylate (50 $mgkg^{-1}$, i.v.) on both mechanical responsiveness and ongoing discharge (see figs. 9.13 - 9.14).

9.2.5 Plasma concentrations of paracetamol and aspirin

In a further six anaesthetized rats, samples of blood were taken 5, 10, 15, 30 and 45 minutes after a single injection of 1-AS (50 $mgkg^{-1}$, ASA equivalent, i.v.) or 45 minutes after a single injection of paracetamol

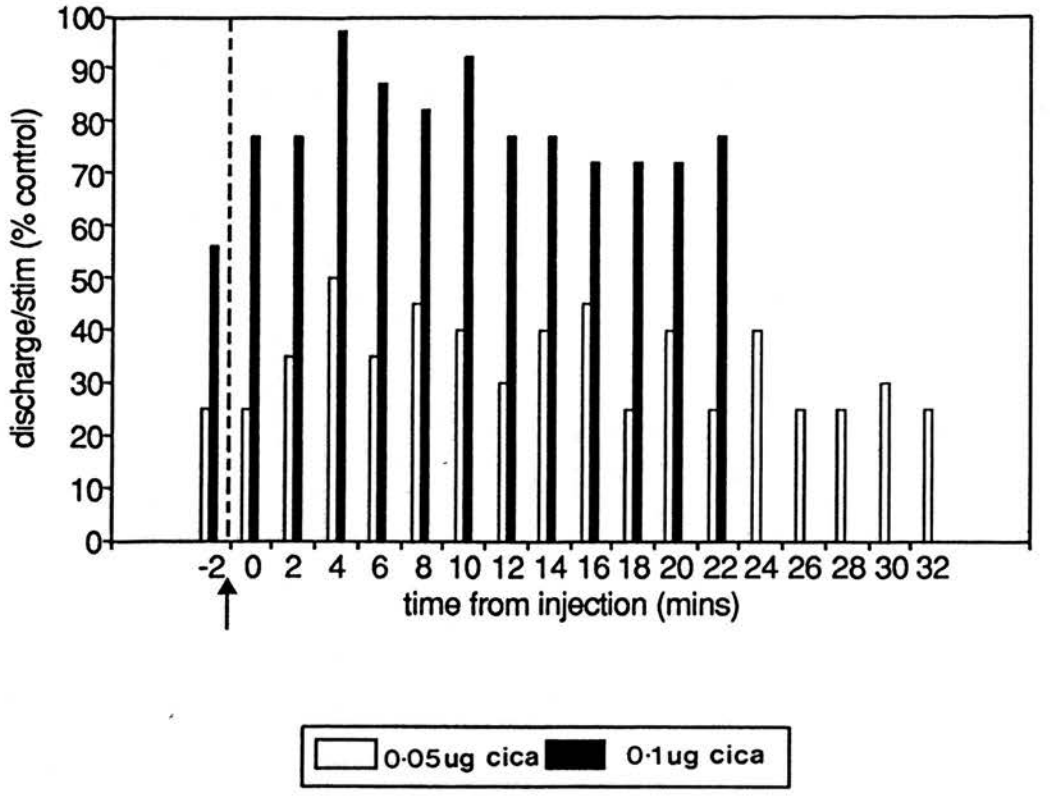

Figure 9.11 Effect of cicaprost on mechanonociceptor responsiveness to mechanical stimuli after paracetamol. Each bar represents the mean response to a standard mechanical stimulus both before and after injection of cicaprost (0.1 and 0.5 μg, i.a. in two separate units). Values are given as a percentage of the pre-paracetamol (50 $mgkg^{-1}$, i.v.) control response.

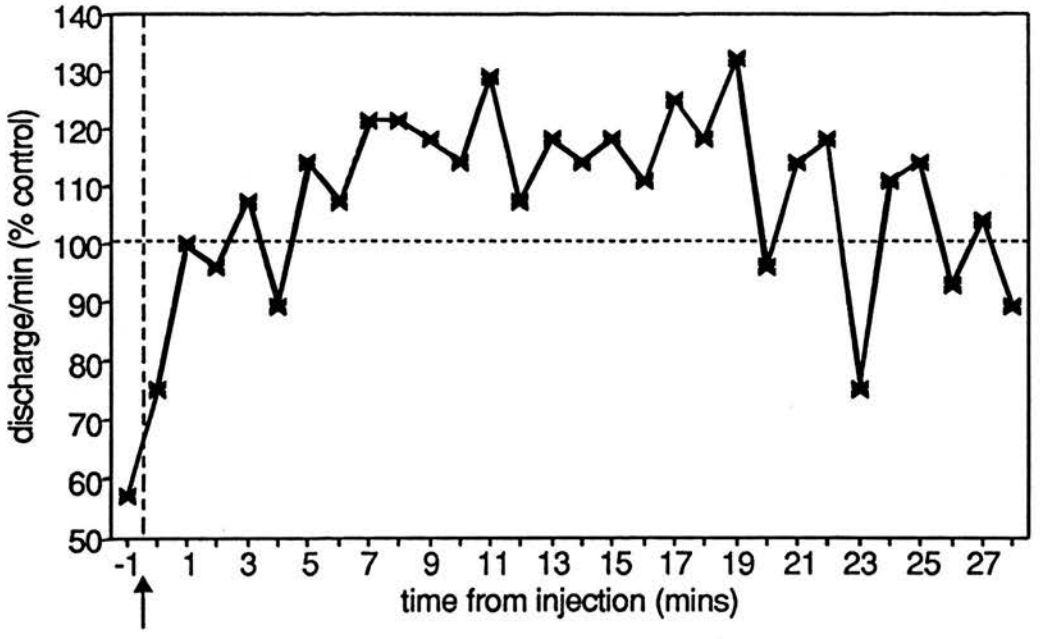

Figure 9.12 Effect of cicaprost on spontaneous mechanonociceptor discharge after reduction by paracetamol. Each point represents the discharge of a single unit, both before and after injection of cicaprost (0.5 μg, i.a.). Values are given as a percentage of the pre-paracetamol (50 $mgkg^{-1}$, i.v.) control discharge (0.5 i.p.s).

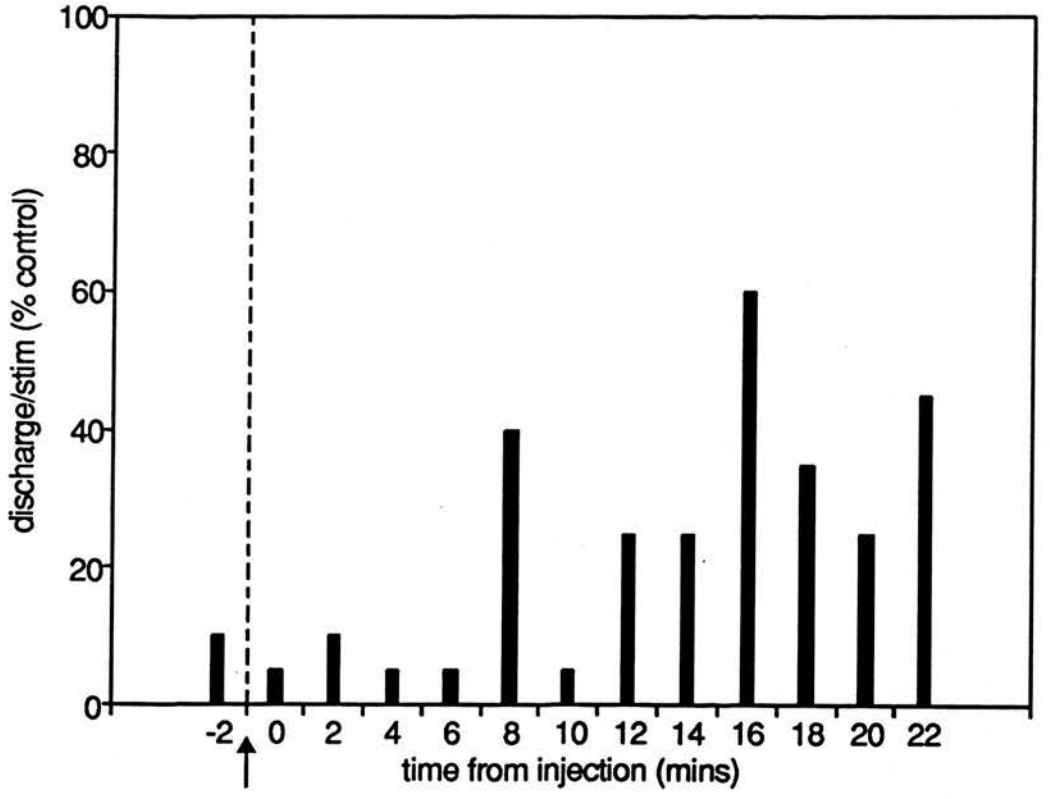

Figure 9.13 Effect of cicaprost on mechanonociceptor responsiveness to mechanical stimuli after sodium salicylate. Each bar represents the response of a single unit to a standard mechanical stimulus both before and after injection of cicaprost (1 μg, i.a.). Values are given as a percentage of the pre-sodium salicylate (50 $mgkg^{-1}$, i.v.) control response.

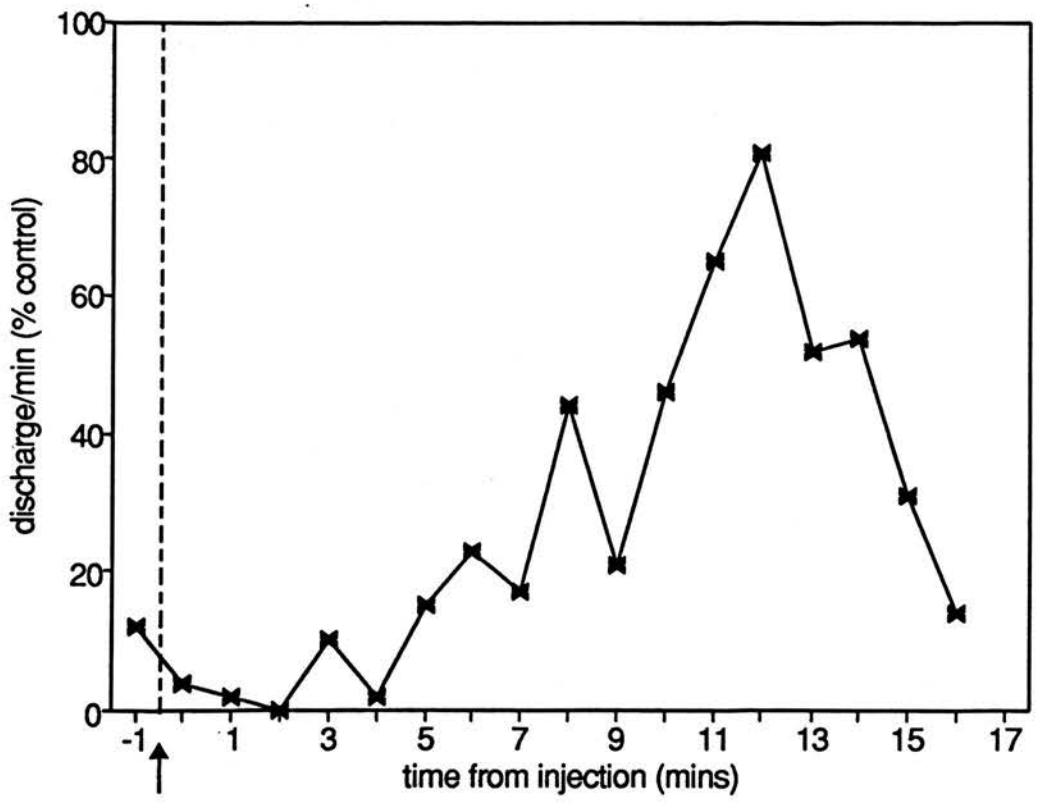

Figure 9.14 Effect of cicaprost on spontaneous mechanonociceptor discharge after reduction by sodium salicylate. Each point represents the discharge of a single unit, both before and after injection of cicaprost (1 μg, i.a.). Values are given as a percentage of the pre-sodium salicylate (50 $mgkg^{-1}$, i.v.) control discharge (0.8 i.p.s.).(50 $mgkg^{-1}$, i.v.). Levels of total drug found in the plasma of the blood samples are shown in table 9.1.

Table 9.1 Plasma salicylate and paracetamol levels following single i.v. injections of 1-AS (50 $mgkg^{-1}$, ASA equvalent) or paracetamol (50 $mgkg^{-1}$).

time after injection (mins)	plasma salicylate (μgml^{-1}) n=3	plasama paracetamol (μgml^{-1}) n=3
5	56.5 ± 11.2	-
10	76 ± 0	-
15	37.2 ± 11.2	-
30	50.1 ± 12.9	-
45	75.9 ± 11.2	16.2 ± 1.3

9.2.6 Effects of 1-AS, sodium salicylate and paracetamol on prostanoid levels of capsular tissue in arthritic joints

In a separate series of experiments thirty five rats with established arthritis were randomly distributed into five equal groups. Individual groups were given twice daily i.p. injections of either 1-AS at 50 or 250 $mgkg^{-1}day^{-1}$ sodium salicylate at 50 or 250 $mgkg^{-1}day^{-1}$, paracetamol at 50 or 250 $mgkg^{-1}day^{-1}$, or saline vehicle (control). Drug dosing was carried out from day 10 post-adjuvant through to day 14. The rats were sacrificed at the end of day 14 and the chronically inflamed ankle joint capsular tissues removed for the subsequent extraction and assessment of tissue prostanoids (see Methods, Section II). Before dosing the solutions were coded and their identities not revealed until the study was concluded.

Results from the analysis of tissue prostanoid content are summarized in figure 9.15. Values from saline control group show that tissue levels of PGE_2 are significantly higher ($p < 0.05$) than those of the PGI_2 measured as its stable hydrolysis product 6-keto $PGF_{1\alpha}$. Both high and low dosage schedules of 1-AS or paracetamol significantly ($p < 0.05$) reduced tissue levels of PGE_2, while only the high dosage of sodium salicylate caused a significant reduction in the levels of this prostanoid. In the case of 6-keto $PGF_{1\alpha}$, tissue levels were significantly reduced by both the low and high doses of 1-AS, but only by the high doses of sodium salicylate or paracetamol.

Blood samples were taken at the end of day 14 from one rat in each group, and the plasma salicylate levels were determined. Results are summarized in table 9.2.

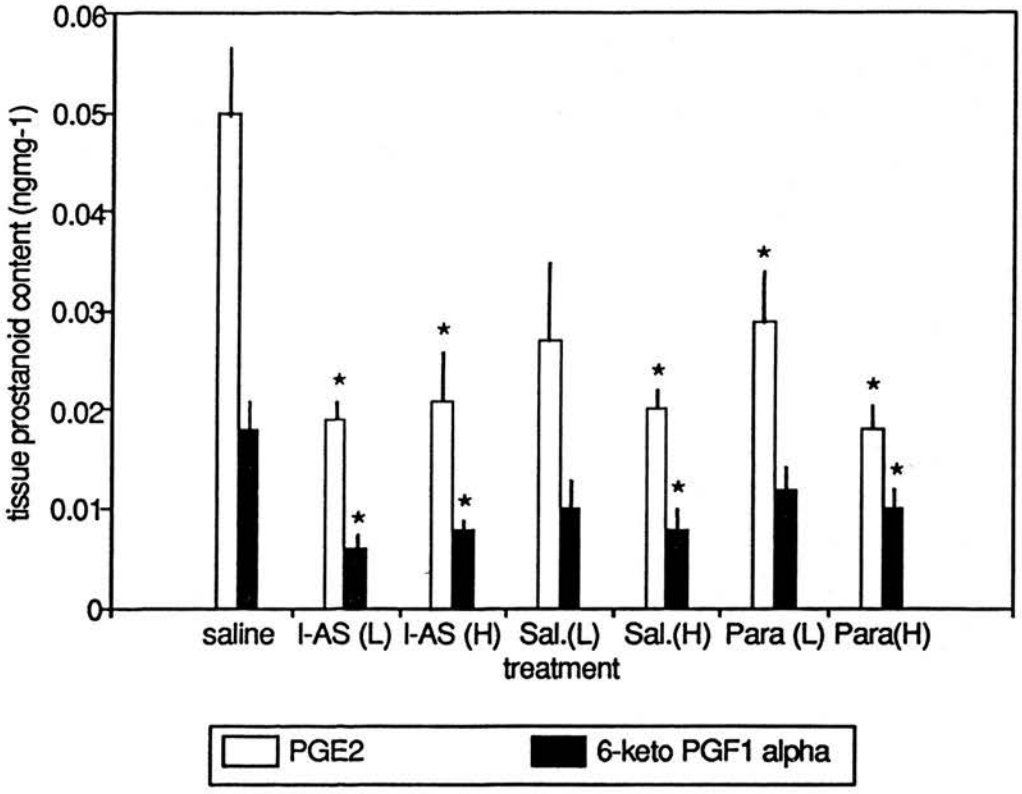

Figure 9.15 Summary of the effects of 1-AS, sodium salicylate or paracetamol on the prostanoid content of chronically inflamed joint capsular tissue. Each bar represents the mean PGE_2 or 6-keto $PGF_{1\alpha}$ content of ankle joint capsular tissue from arthritic rats given daily doses of 1-AS (50 or 250 $mgkg^{-1}day^{-1}$, i.p.), sodium salicylate (50 or 250 $mgkg^{-1}day^{-1}$, i.p.) or paracetamol (50 or 250 $mgkg^{-1}day^{-1}$, i.p.). The letters L and H denote the low and high doses respectively. The vertical bars represent the s.e.m.. For each group, including the saline control n=5. Values which are significantly different from those in saline (control) treated rats are shown as *, when $p < 0.05$ (Students T-Test).

Table 9.2 Plasma salicylate levels measured after five days of daily dosing with l-AS (50 or 250 $mgkg^{-1}$ day^{-1}, ASA equivalent, i.p.) or sodium salicylate (50 or 250 $mgkg^{-1}$ day^{-1}, i.p.).

drug treatment	plasma salicylate (μgml^{-1})
l-AS (50 $mgkg^{-1}day^{-1}$)	14.6
l-AS (250 $mgkg^{-1}day^{-1}$)	250.2
sod.sal. (50 $mgkg^{-1}day^{-1}$)	14.67
sod.sal. (250 $mgkg^{-1}day^{-1}$)	112.8

9.3 DISCUSSION

Results from electrophysiological experiments using 1-AS confirm reports showing that aspirin reduces both the mechanical sensitivity and the ongoing discharge of articular mechanonociceptors from chronically inflamed rat ankle joints (Guilbaud & Iggo, 1985). These findings support the idea, first proposed by Lim et al. (1964), that the analgesic action of ASA is mediated via a peripheral site. Furthermore, in the present study, the depressant action of 1-AS was shared by sodium salicylate and paracetamol, suggesting that these drugs also have an analgesic action mediated via a peripheral site associated with nociceptive sensory receptors in the joint tissues.

ASA has been shown to inhibit prostanoid synthesis both *in vitro* (Vane, 1971; Ferreira & Vane, 1974; Roth et al., 1975; Sturge et al. 1978; Van der Ouderas et al., 1980; Salmon et al., 1983), and *in vivo* (Ferreira & Vane, 1974; Moncada et al., 1975; Fitzpatrick & Wynalda, 1976), and is considered to exert its analgesic effects via the resulting reduction in tissue prostanoid content (Collier et al., 1972; Ferreira & Vane, 1974; Moncada et al., 1975). Results detailed in Section VII of this thesis, showing that the depressant effects of 1-AS in arthritic joints can be overcome by the administration of exogenous prostanoids, suggest that these effects are mediated via the inhibition of endogenous prostanoid production. This effect of exogenous prostanoids was also seen in the present study following sodium salicylate-induced depression of mechanonociceptor discharge. Although sodium salicylate is a poor inhibitor of cyclooxygenase *in vitro* (Flower & Vane, 1972), when administered *in vivo* salicylates effectively

decrease the content of prostaglandins in inflammatory exudates (Willis et al., 1972; Higgs et al., 1976). Because 1-AS or sodium salicylate reduced the PGE^2 and 6-keto $PGF_{1}\alpha$ content of chronically inflamed joint capsular tissue it is likely that both 1-AS and sodium salicylate are acting to depress mechanonociceptor discharge via a reduction in endogenous prostanoids. Furthermore, this reduction in tissue prostanoid levels was achieved with similar blood plasma concentrations to that affecting mechanonociceptor discharge.

ASA is known to irreversibly acetylate the active site of cyclooxygenase (Roth et.al., 1975). However, in vivo acetylsalicylate is subject to conversion by the liver to salicylate (Cerletti et al., 1985), and inhibition of cyclooxygenase activity may be explained by the combination of salicylate with cyclooxygenase. It has been suggested that NSAID drugs interact with an enzymic receptor (Scherrer, 1974; Gund & Shen, 1977), and more recently that NSAIDS may interact at two different sites on the cyclooxygenase enzyme - the catalytic site and the so called 'supplementary' site (Humes et al, 1981). Thus, it has been claimed that potent inhibitors interact with both sites, while weak inhibitors, such as salicylate, act more at the supplementary site. Sodium salicylate has about the same potency as ASA in anti-pyretic and anti-inflammatory tests (Collier, 1969), suggesting that at least some of the actions of ASA are shared by salicylate. Although the evidence for a mechanism of action of NSAIDS through inhibition of cyclooxygenase is very strong, it should also be noted that several other enzymes are affected by these drugs (Lembeck & Juan, 1974; Tolman and Partidge, 1975; Kuehl & Egan, 1980; Brune, 1982; 1983), and that anti-inflammatory doses of NSAIDS are known to inhibit neutrophil activation (Abramson,

1985), providing a possible explanation for their anti-inflammatory activity.

In the present study the demonstration that paracetamol reduces mechanonociceptor discharge and mechanical sensitivity in arthritic joints, together with the finding that this effect can be reversed by the administration the PGI_2 analogue, cicaprost, suggests that this action is mediated via a reduction in exogenous prostanoids in the periphery. Further support for this suggestion is provided by results showing that paracetamol causes a reduction in levels of PGE_2 and 6-keto $PGF_{1\alpha}$ in chronically inflamed joint capsular tissue. Paracetamol is also known to reduce prostanoid levels in inflammatory exudates (Higgs et.al., 1976), and, furthermore, has been shown to possess anti-inflammatory activity in man (McQueen, 1973; Skjelbred et.al., 1977), and in rats (Vinegar et.al., 1976; Wong & Gardocki, 1983). It has been shown that paracetamol selectively inhibits nervous tissue cyclooxygenase with little effect on enzymic preparations from other tissues (Flower & Vane, 1972; Dembinska-Kiec et.al., 1976). Flower & Vane (1972) proposed that the analgesic effect of paracetamol was due to its selective action on nervous tissue prostaglandin synthetase enzymes, thus indicating a predominantly central site of action. However, studies by Ferreira et. al. (1978), suggested that paracetamol had both centrally and peripherally mediated analgesic effects. Based on their knowledge of its selective action on nervous tissue cyclooxygenase, Ferreira and colleagues (1978) proposed that this peripheral effect could be at the nociceptive sensory ending. Recent evidence suggets that a further peripheral target for a nervous tissue specific cyclooxygenase inhibitor may be sympathetic postganglionic nerve terminals associated

with nociceptive sensory endings (Levine et.al., 1986). However, studies refuting evidence for heterogeneity, and differential sensitivity of cyclooxygenase enzymes in the same species have also been reported (Pong & Levine, 1976). In the present study the additional depressant effects of 1-AS seen following paracetamol indicate that the two drugs may be acting by some differing mechanism. This may be explained in terms of an additional action of 1-AS via the irreversible acetylation of cyclooxygenase. An additional complication is provided by the finding that paracetamol only weakly inhibits purified cyclooxygenase when tested under standard assay conditions, in which the peroxide concentration may rise to a high level, and paracetamols potency as a cyclooxygenase inhibitor is increased if the peroxide concentration is reduced to levels found intracellularly in vivo (see Lands, 1981). High levels of peroxide seen during inflammation (see Blake et al., 1987) may explain the generally reported weak anti-inflammatory effect of paracetamol.

In contrast to the side-effects associated with NSAID drugs (Lifschitz, 1983; O'Brien & Bugby, 1985; Adams et. al.,1986; Goodwin, 1987), paracetamol has few side effects, apart from hepatic cellular necrosis which mainly occurs during overdosage (Prescott, 1983). A large number the side effects of NSAIDS have been attributed to inhibition of prostanoid production in peripheral tissue (Vane, 1971), supporting the proposal of a selective action of paracetamol on nervous tissue cyclooxygenase. If this were the case then the reduction in the prostanoid content of inflammatory exudates (Higgs et.al.1976), and chronically inflamed tissues caused by paracetamol, would have to be a result of actions on peripheral nervous tissue. Depression of

excitability in C fibre afferents may be expected to reduce neurogenic components of inflammation (see Holzer, 1988) through a reduction in the peripheral release of neuropeptides such as substance P and CGRP, and as a consequence of this would cause a reduction in the production of inflammatory prostanoids. In this way reductions in tissue prostanoid content seen in the present study could be explained.

9.3 CONCLUSIONS

It has been demonstrated that 1-AS, sodium salicylate and paracetamol depress the activity of sensitized articular mechanonociceptors from chronically inflamed ankle joint tissues. These findings support the idea that these drugs exert their analgesic effects at least partly through a peripheral site of action. This peripheral analgesic effect may be mediated via a reduction in endogenous prostanoids as it has also been shown that (a) these depressant effects can be at least partially reversed by the administration of exogenous prostanoids, and (b) that these drugs are capable of reducing tissue prostanoid content in arthritic joints. Although the particular mechanism of action needs to be elucidated, the results presented, together with evidence that paracetamol has anti-inflammatory properties (McQueen, 1973; Vinegar et.al., 1976; Skjelbred et.al.,1977; Wong & Gardocki, 1983), suggest that paracetamol may be of use as an adjunct to conventional NSAID therapy in the treatment of arthritis in man. In addition high doses of NSAIDS used to increase the level of pain relief have not been found to show any greater anti-inflammatory effect in rheumatoid arthritis (Orme

et.al., 1976; Grennan et.al., 1973; Dunagan et.al., 1988; Seideman & Melander, 1988). The large number of side effects associated with NSAIDS, and the concept that total blockade of prostanoid production may in fact increase joint pathology, either by increasing production of lypoxygenase products of arachidonic acid (Walker & Harvey, 1984) or reducting in anti-inflammatory prostanoids (Cammarata & Aspinall, 1969; Zurier, 1971), suggests that the use of analgesics such as paracetamol may be desirable.

SECTION X

GENERAL DISCUSSION AND CONCLUSIONS

SECTION X

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The involvement of various endogenous chemical mediators in the increased nociceptive activity seen in chronically inflamed joints was investigated using a novel model of localized adjuvant-induced arthritis in the rat. Results from these studies can be used to define the relative importance of these mediators in causing the joint pain associated with arthritis.

Localized adjuvant-induced monoarthritis as a model for the neuropharmacological study of chronic inflammation in rats

Relatively low doses of F.C.A., injected subdermally around the tibio tarsal joint, induced an arthritis which was localized to the ankle region of one hindlimb. The time course of over which tissue swelling and hyperalgesia developed followed a similar pattern found by Stein et al. (1988) using a model of adjuvant-induced inflammation of one hindpaw. The bilateral nature of adjuvant-induced polyarthritis has been suggested to result at least partly through a contribution from the nervous system. Basbaum et al. (1988) concluded that several components of the nervous system contribute to the inflammatory process producing joint injury in experimental arthritis. These include the peripheral terminals of both small-diameter peptide containing primary afferents

and sympathetic efferents, large diameter primary afferents, and descending brainstem inhibitory control systems. However, in the present series of studies no evidence of contralateral inflammation was obtained. Furthermore, results from other investigations using localized forms of chronic inflammation in the rat have described only unilateral increases in opioid peptides in the dorsal horn of the spinal cord (Stein et al., 1987), and increases in levels of CGRP found in primary afferents (Nahin et al., 1990) or dorsal root ganglia (Smith et al., 1990). Although the nervous system probably has role in inflammation, these findings and those from the present study indicate that the bilateral nature of adjuvant polyarthritis may be due to factors other than those associated with the nervous system. Antigenic or humoral factors released during inflammation may be responsible. The low dose of adjuvant used in the present study, together with the mild nature of the inflammation probably results in fewer of these factors being released to affect contralateral limb. Further investigations using higher doses of adjuvant to determine a threshold for bilateral effects, together with studies examining alterations in nociceptive sensory systems will be required to determine the precise role played by the nervous system in bilateral chronic inflammatory joint disease. These studies should include examination of the changes in neuropeptide levels taking place in primary afferents and spinal dorsal horn neurones, and the mechanisms by which these changes occur. The behaviour of second order neurones in the spinal cord and the activation of reflex pathways during chronic inflammation should also be examined.

The limited nature of the bone and cartilage destruction described in the present model differs from the severe erosion seen in adjuvant polyarthritis, and more closely resembles the rheumatoid disease in humans (see Rainsford, 1982). Adjuvant-induced arthritis appears to be a particularly useful model, and is considered to be the most appropriate for the pharmacological study of the pain associated with clinical arthritis. The development of this new localized model of adjuvant arthritis provides considerable benefits, both on scientific and ethical grounds. Studies using the model are not complicated by the severe systemic changes seen in adjuvant polyarthritis, and furthermore provide the option of using the contralateral unaffected joint as a within the same animal control.

Electrophysiological investigations in arthritic joints showed that there was a large increase in the number of identifiable receptive fields for articular mechanonociceptors compared to normal joints. Mechanonociceptors had lower thresholds for mechanical activation, and generally displayed a resting discharge not seen in normal joints. These results are in agreement with those of several other workers using models of both chronic (Guilbaud et al., 1985), or acute (Coggeshall et al., 1983; Grigg et al., 1986; Schaible and Schmidt, 1985; 1988) inflammation of the joints. Mechanonociceptors in rat arthritic ankle joints were also found to be more sensitive to intra-arterial injections of chemical excitants such as capsaicin or 5-HT. In addition to the identified mechanonociceptor activity, high levels of ongoing discharge were seen in afferent units for which no mechanosensitive receptive fields could be found in the joint tissues. Chemosensitive units were found to be excited by chemical stimuli including capsaicin, the

selective excitant of fine afferent fibres. Selectively chemosensitive sensory receptors which become active during inflammation have also been described by other workers (see McMahon & Koltzenburg, 1990). These findings and those of the present study are extremely important and provide a means whereby total nociceptive input to the spinal cord and higher perceptual centres can be greatly increased during arthritis. The sensory receptors described here do not fit into the classical definition of a nociceptor, and are perhaps best described as inflammation receptors (Iggo, 1988), signalling the chemical microenvironment of peripheral tissue. This hypothesis provides an explanation for the long unanswered question of why there are proportionately so many unmyelinated afferents innervating peripheral tissue.

In the present study identification of the receptive fields and afferent fibre conduction velocities of chemosensitive units was not fully investigated. Although no mechanically sensitive receptive fields could be found for these units by probing on the joint surface, this does not exclude the possibility that they were not responsive to mechanical stimuli. Identification of receptive fields for specifically chemosensitive units has been accomplished by Meyer and Campbell (1988) in primate skin using a localized electrical stimulus as a probe. This approach should be considered in future studies on rat ankle joints to determine if the units described here are in fact selectively chemosensitive.

The rat isolated hindlimb preparation

A novel isolated hindlimb preparation was developed for use in neuropharmacological studies *in vitro*. Mechanonociceptors from normal ankle joints displayed similar characteristics to those seen *in vivo*. Identified C fibre units had high mechanical activation thresholds, displayed slowly adapting responses to a mechanical stimulus of constant amplitude, and were excited and sensitized by chemical stimuli. These are typical properties of sensory receptors, and thus, do not appear to be dependent upon blood-borne factors or an intact sympathetic innervation. Mechanical and chemical responsiveness was observed for periods of up to eight hours after removal of the limb, suggesting that the ionic balance and oxygen content of the tissue around the sensory endings is sufficient to sustain normal receptor function.

Electrophysiological studies on arthritic joints *in vitro* revealed the presence of increased numbers of mechanonociceptor receptive fields, as seen *in vivo*. Lower thresholds for mechanical activation and higher levels of resting discharge were also characteristic of arthritic joints. These findings indicate that the enhanced mechanonociceptor sensitivity is not dependent on the presence of blood constituents, nor on CNS influences.

The fact that normal sensory receptor function can be seen over prolonged periods, together with the increased sensitivity of sensory receptors in arthritic joints, suggests that the isolated hind limb preparation could be used in neuropharmacological studies examining the effects of chronic joint inflammation on nociceptor responsiveness.

Furthermore, the isolated nature of this preparation makes it ideal for using two limbs from the same animal in order to examine sensory receptors in the athritic and contralateral unaffected joints.

A number of other workers have also successfully developed preparations for the study of sensory receptors in vitro. Carotid body chemoreceptors (Eyzaguirre & Lewin, 1961), superfused diaphragm-phrenic nerve (Kieschke & Mense, 1984), canine testis-spermatic nerve (Kumazawa & Mizumura, 1983), isolated perfused rabbit ear-auricular nerve (King et al. 1976) and rat skin in vitro (Reeh, 1986) preparations have been examined. All of these preparations provide the advantage of the experimenter being able to control the environmental conditions in the tissue. Concentrations of exogenous chemical can be controlled either through bath application or by direct application to the receptive fields of single units. In the present study drugs were injected into the perfusate, and reached their target sensory receptors via the vasculature supply of the limb. The possibility of applying drugs more locally to single receptive fields in the rat isolated hindlimb preparation has also been suggested. It would be possible to apply discrete and quantifiable amounts of solution to single receptive fields within the joint tissue by pressure ejection using a glass micropipette. This technique was attempted during the course of my work, but insufficient time was available to produce any meaningful data. Further studies using this technique of drug administration may provide more accurate measure of concentration related effects than were obtained in the present study.

5-Hydroxytryptamine

Intra-arterial injections of 5-HT excited and sensitized articular receptors from normal and arthritic joints. Sensory receptors in arthritic joints were more sensitive than those from normal joints. Rapid excitatory events are most likely to result from a direct action of the amine at 5-HT receptors associated with sensory terminals within the joint tissues. However, delayed excitation and mechanonociceptor sensitization may occur secondary to the local release of other endogenous excitants or sensitizing chemicals such as bradykinin or the prostanoids. In support of a direct action on 5-HT receptors associated with sensory terminals are the findings that 5-HT has delayed effects on isolated sensory neuronal preparations in vitro (Higashi, 1977; 1980; Simmonds & DeGroat, 1980; Wallis et al., 1982; Christiansen et al., 1982). These delayed nature of these effects is likely to be due to 5-HT receptor mediated activation of intracellular second messengers such as IP3 or cAMP (see Peroutka, 1988). The present findings are in keeping with existing knowledge on the actions of 5-HT. In neuronal cells 5-HT activates a wide range of conductances either via receptor gated ion channels (5-HT_3) or through activation of second messenger systems (ie 5-HT_2). An individual cell often has more than one response to a single application of 5-HT, these often being separated by their time course. This variety of actions is due, at least in part, to the diversity of receptor subtypes and their coupling to distinct second messenger systems and ion channnels. Further investigations into the role of

second messengers in 5-HT mediated effects on sensory receptors will be required if the excitatory and sensitizing actions of the amine are to be completely understood.

In arthritic joints administration of 5-HT_2 or 5-HT_3 receptor antagonists caused short lasting reductions in resting discharge, supporting a role for 5-HT in the maintenance of the high levels of activity seen in these joints. Likely sources of endogenous 5-HT in the rat are blood platelets (Garratini & Valzelli, 1965; Franzen & Eysell, 1969), mast cells (Johnson & Erdos, 1973), or sympathetic neurones (Dun et al., 1980; Verhofsted et al., 1981; Neel & Parsons, 1986). Since 5-HT is not found in human mast cells, it has not generally been considered to be an important mediator of inflammation in humans. However, blood platelets may release 5-HT during immune activation or as a result of tissue damage, and have recently been implicated in the progression of a number of inflammatory diseases (see Page, 1988). It has also been suggested that the sympathetic nervous system may have a role in the development of pain in arthritis (see Fitzgerald, 1989). Thus, over-activity of the sympathetic nervous system during inflammation may result in an increased release of 5-HT from sympathetic terminals. Although a 5-HT_3 receptor has been implicated in 5-HT-induced pain in the human blister base (Fastier et al., 1959; Donatsch et al, 1984b; Richardson et al, 1985), the involvement of a 5-HT_2 receptor in delayed nociceptive responses has not been reported previously. Further investigations into the role of 5-HT_2 receptors in the pain associated with chronic inflammation is warranted in view of the present findings.

Bradykinin

Intra-arterial injection of bradykinin excited and sensitized articular mechanonociceptors to mechanical stimuli in both normal and arthritic joints. Results from this study and those of other workers suggest that the excitatory effects of bradykinin are at least partially dependent upon the sensitizing actions of other substances such as 5-HT (Mense, 1981), or the prostanoids (Chahl & Iggo, 1977; Kumazawa & Mizumura, 1980; Mense, 1981; 1982). The excitatory effects of bradykinin on nociceptive sensory receptors in normal tissues is mediated via a B2 receptor (Mizumura et al., 1990). In further studies characterization of bradykinin receptors mediating the effects seen in the present study would be of interest, particularly in arthritic rats, as the B1 receptor is known to be synthesized during inflammation (Regoli et al., 1978; Marceau et al., 1980).

Production of bradykinin is markedly increased during inflammation (see Lewis, 1970; Garcia-Leme, 1978), and a number of pro-inflammatory responses are triggered by bradykinin (Regoli & Barabe, 1980; Manning et al., 1982; Marceau et al., 1983; Rozengurt, 1986). However, during chronic inflammation many actions of bradykinin, including the induction of pain, are likely to be greatly potentiated by other inflammatory mediators such as the prostanoids. It has been suggested that the development of selective bradykinin receptor antagonists may be useful in the treatment of the pain associated with inflammation. However, it may be more beneficial block the effects of the potentiating substances rather than the acute and perhaps protective pro-inflammatory functions of bradykinin using receptor antagonists.

Prostanoids

Intra-arterial injection of PGE_2, PGI_2, or the selective IP-receptor agonist cicaprost, excited and sensitized articular mechanonociceptors to mechanical stimuli. These prostanoids also potentiated the effects of bradykinin on articular sensory receptors. Examination of the effects of PGE_2, PGI_2, and cicaprost *in vivo*, or PGE_2, cicaprost, PGD_2, and $PGF_{2\alpha}$ *in vitro*, produced the following rank order of potency

$$PGI_2 = \text{cicaprost} \gg PGE_2 \gg PGD_2 = PGF_{2\alpha}$$

A similar order of potency was obtained for prostanoid-induced depolarization of the rat or rabbit isolated vagus nerve *in vitro*, with, in addition, PGE_1 being equipotent with PGI_2, and the TXA_2 mimetic U46619 being inactive. These results are in agreement with previous reports showing that PGI_2 and PGE_1, which has potent agonist activity both at IP- and EP-receptors (Coleman et al., 1987), are more potent hyperalgesic agents than PGE_2 (Juan, 1979). The present findings provide evidence for the involvement of IP-, and perhaps EP-receptors, in the excitatory and sensitizing actions of the prostanoids on articular nociceptors in the rat. In view of the general concensus that prostanoids do not cause overt pain when administered alone, the present finding that i.a. PGI_2 or cicaprost excited articular nociceptors is particularly novel. In humans, prostanoid-induced pain is only reported when relatively high doses of PGE_1 are administered intradermally, whereas low doses of PGE_1 potentiate the pain caused by bradykinin (Ferreira, 1972). It cannot be discounted that the excitatory action of PGI_2 seen in the present study resulted from IP-receptor mediated sensitization to an endogenous excitant already present in the joint

tissues. Indeed excitation of articular nociceptors was caused by the combined i.a. injection of subthreshold doses of PGI_2 and bradykinin. However, in studies on rat isolated sensory neurones PGE_2 causes these cells to fire action potentials (Baccaglini & Hogan, 1983), suggesting that the prostanoids can directly excite nociceptive neurones in the absence of other mediators such as bradykinin.

The use of isolated nerve preparations in order to determine which prostanoid receptors may be present on sensory terminals has proved to be successful in the present study. Investigations using isolated nerve preparations have contributed significantly to the development of selective antagonists for neuronal 5-HT receptors (see Ireland, 1987; Ireland and Tyers, 1987; Butler et al., 1988), and should be useful in further studies on the prostanoids. Prostanoid-induced depolarization of fine sensory nerves from the rat and rabbit was shown to be mediated predominantly by IP-receptors. The prostanoid receptor mediating PGE_2-induced depolarization of the rabbit isolated vagus could not be determined using the standard available selective agonists for the EP_1-, EP_2- and EP_3-receptors. Further studies will be necessary to determine if the receptor mediating this response is a novel EP-receptor subtype. However, the development of new selective agonist and antagonist compounds for prostanoid receptors will be required before further advances can be made.

Results obtained in the present study examining the possible involvement of second messenger systems in prostanoid-induced depolarization of the rabbit vagus are in agreement with previous findings showing that in sensory neurones the effects of prostanoids are mediated via the activation of cAMP (Kalix, 1979; Gilman et al., 1971;

Fowler et al., 1985a,b; Weinreich and Wonderlin, 1987). Prostanoid-induced hyperalgesia has also been postulated to be mediated via cAMP (Ferreira & Nakamura, 1979). Further investigations examining the effects of non-hydrolysable analogues of cAMP and forkolin on sensory receptors would be of use in determining whether these second messengers have sensitizing and excitatory effects similar to those of the prostanoids. The rat isolated hindlimb preparation would be of most use in this type of study, since a greater level of control could be achieved over the environment to which the drugs are added.

Results from the present study examining the effects of 1-AS, PGE_2 and cicaprost support the hypothesis, first proposed by Guilbaud & Iggo (1985), that PGI_2 is the major endogenous prostanoid resposible for enhancing mechanonociceptor sensitivity in arthritic rat ankle joints. Determination of whether the effects of 1-AS seen in the present study is solely due to a supression of PGI_2 production awaits the development of selective IP-receptor antagonists. There is evidence to support a role for PGI_2 in human rheumatoid arthritis, since an extensive study examining the prostanoid content of synovial fluid from human arthritic joints found 6-keto-$PGF_{1\alpha}$, the stable breakdown product of PGI_2, to be present at the highest concentrations (Brodie et al., 1980).

Prostanoids may be produced by a variety of different cell types during inflammation, including platelets (Willis, 1978), mast cells (Becker & Henson, 1973; Roberts et al., 1978), neutrophils (Goldstein et al., 1977; Zurier, 1976), mononuclear phagocytes (Davies & Allinson, 1976) and macrophages (Gordon et al., 1976; Myatt et al., 1976; Humes et al., 1977). Prostanoids are produced by inflamed tissues ex vivo, such as cultured human synovium which produces PGE_2 (Robinson et al., 1975),

PGI_2 and TXA_2 (Salmon et al., 1983). In addition it has recently been suggested that sympathetic neurones synthesize and release prostanoids from their postganglionic terminals (Taiwo & Levine, 1988), and that the contribution made by the sympathetic nervous system to arthritic pain (see Fitzgerald, 1989) is mediated through the actions of these prostanoids on nociceptive sensory terminals.

The potential of using selective prostanoid receptor antagonists as analgesics in human rheumatoid disease may provide considerable advantages over conventional NSAID analgesic therapy. The blockade of prostanoid production by NSAIDS is predominantly responsible for the high incidence of side effects associated with their use in the treatment of rheumatoid arthritis (Lifschitz, 1983; Goodwin, 1987). NSAIDS may also potentiate chronic inflammation (Lewis & Barrett, 1986), and have a harmful effect on cartilage (Palmoski & Brandt, 1985). It has been suggested that these effects may result from an increased production of lipoxygenase products of arachidonic acid metabolism (Walker & Harvey, 1984) or through loss of the known anti-inflammatory effects of the prostanoids themselves (Aspinall & Cammarata, 1969; Lichtenstein & DiBernard, 1971; Orange et al., 1971; Zurier & Quagliata, 1972; Glenn & Rohloff, 1972; Zurier et al., 1972; 1974; Bonta et al., 1978;). By using selective prostanoid antagonists it may be possible to provide analgesic and anti-inflammatory activity without the adverse effects caused by the total blockade of prostanoid production.

Conclusions

Overall the results presented support a role for bradykinin, 5-HT and the prostanoids PGI_2 and PGE_2 in the pain associated with chronic joint inflammation in the rat. More than one mediator is likely to be responsible for the sensitization of articular nociceptors seen in arthritic rat ankle joints. As modulators of the responsiveness of nociceptive sensory receptors to other mediators, and as potent excitants themselves, the prostanoids, and in particular PGI_2, probably play a major role in sensitizing nociceptors in arthritic joints. The potent analgesic effects of NSAIDS in man suggests that the prostanoids are important mediators of pain in human joint disease with the prostanoids PGE_2 and PGI_2 likely to be most relevant to humans since levels of these prostanoids are high in synovial fluid from patients with rheumatoid arthritis (Brodie et al., 1980). In addition to the prostanoids, future investigations should consider the actions of other endogenous chemical mediators on nociceptive sensory nerves. Of particular interest is the suggested sensitizing action of 8(R),15(S)-dihydroxyeicosatetraenoic acid (8(R),15(S) diHETE) via a direct effect on sensory terminals (Taiwo et al., 1987), and the proposed peripherally mediated analgesic effects of the opioids during inflammation (Ferreira & Nakamura, 1979; Hargreaves et al., 1988; Stein et al, 1988).

With the recent discovery of a host of putative inflammatory mediators such as the cytokines, platelet activating factor and various neuropeptides (see Willougby, 1987), our picture of chronic inflammatory process is becoming increasingly complex. The mediators involved in the pain associated with inflammation and their mechanisms of action are

only now starting to emerge. It is likely to take a considerable amount of additional time and effort before these processes are fully understood, and new potent analgesic, anti-inflammatory compounds are developed.

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APPENDIX I

DETAILS AND SOURCES OF DRUG USED IN THIS INVESTIGATION

Drug	Source
4-Acetomidophenol (paracetamol)	Sigma
AH13205	Glaxo, UK
Bradykinin, acetate salt	Sigma
Capsaicin (8-methyl-n-vanylyl-nonenamide)	Sigma
Cicaprost (ZK 96480)	Schering, A.G. Berlin
GR38032F (1,2,3,9-tetrahydro-9-methyl-3-[2-methyl-1H-imidaxol-1-yl)methyl]-4-carbazol-4-one hydrochloric dihydrate	Glaxo, UK
5-hydroxytryptamine creatinine sulphate complex, dopamine hydrochloride	Sigma
ICI 81008	ICI Pharmaceuticals Division
ICS 205-930 ((3α-tropanyl)-3yl)-1H-indole-3-carboxylic acid ester)	Sandoz, Basel
Iloprost (ZK 36374)	Schering, A.G. Berlin
Lysine acetylsalicylate	SYLATEC, France
Ketanserin, tartaric acid salt	Janssen Pharmaceuticals, Beerse
MDL 72222 ((1αH, 3α, 5αH-tropan-3-yl) 3,5-dichlorobenzoate methanosulphonate salt)	Merrell Dow, Strasbourg
PGD_2	Glaxo, UK
PGE_1	Glaxo, UK
PGE_2	Glaxo, UK
$PGF_{2\alpha}$	Glaxo, UK
PGI_2	Glaxo, UK
Rioprostil	Glaxo, UK
Sulprostone	Glaxo, UK
U 46619	Upjohn Diagnostics, U.S.A.

APPENDIX II

Br. J. Pharmacol. (1990), **101**, 715–721

The effects of 5-HT on articular sensory receptors in normal and arthritic rats

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1 The effects of intra arterial (i.a.) injections of 5-hydroxytryptamine (5-HT, 1–100 μg) on the discharge of (a) identified articular high threshold mechanoreceptors and (b) unidentified chemosensitive receptors in the ankle joint have been studied electrophysiologically in anaesthetized normal and arthritic rats. Recordings were made from a fine branch of the medial plantar nerve.

2 5-HT increased the mechanical responsiveness of high threshold nociceptive mechanoreceptors with C and Aδ fibre afferents in both normal and adjuvant-arthritic rats. Receptors in arthritic joints were more sensitive to 5-HT than were those from normal joints.

3 5-HT produced a complex response from both types of articular receptors following i.a. injection. Two separate components were identified: (a) a fast transient burst of activity was obtained within 10 s of this injection in 66% of units from normal animals and 45% from arthritics, followed by (b) a delayed slow longer-lasting excitation seen in 62% of the units examined from normals and 77% of units from arthritic rats.

4 Increased mechanoreceptor resonsiveness produced by 5-HT was reduced or abolished by the 5-HT_3 receptor antagonists studied (MDL 72222, ICS 205-930, or GR 38032F, in single doses of 100 $\mu g\,kg^{-1}$, i.a.).

5 Fast excitation showed marked tachyphylaxis and was antagonized by MDL 72222, ICS 205-930 or GR 38032F. It was unaffected by ketanserin (100 $\mu g\,kg^{-1}$, i.a.). Delayed excitation was reduced or abolished by ketanserin but was unaffected by the 5-HT_3-receptor antagonists.

6 Administration of MDL 72222, ICS 205-930 or GR 38032F caused short lasting (< 5 min) reductions in background activity from both types of unit recorded in arthritic rats, as well as in normal rats in which activity had increased following administration of 5-HT. Ketanserin caused similar reductions in background activity in chemosensitive units, but had no effect on mechanoreceptors.

7 At least two types of receptor are involved in the actions of 5-HT on articular sensory receptors with fine afferent fibres. Increased mechano-responsiveness involves a 5-HT_3-receptor as does fast excitation. Delayed excitation probably involves a 5-HT_2-receptor. Endogenous 5-HT appears not to play a crucial role in sensitization of high threshold mechanoreceptors in this model of chronic inflammation and arthritis, although its local release may potentiate the actions of other inflammatory mediators on sensory receptors in the ankle joint.

Introduction

Adjuvant-induced polyarthritis in rats has been used extensively as a model for the study of chronic inflammatory pain (see Colpaert, 1987). Electrophysiological studies with this model have shown that high threshold C-fibre mechanoreceptors (putative nociceptors) have lower thresholds in the ankle joints of these animals in comparison with normal rats (Guilbaud *et al.*, 1985). These results suggest that the behavioural changes seen in adjuvant polyarthritis can partly be accounted for in terms of altered properties of articular sensory receptors. The enhanced receptor sensitivity can be reduced by lysine acetylsalicylate, suggesting that locally produced cyclo-oxygenase metabolites may be responsible for at least part of the sensitization (Guilbaud & Iggo, 1985). It is still uncertain, however, the extent to which other inflammatory mediators found in tissue exudates may contribute to the sensitization of peripheral sensory receptor mechanisms.

Keele & Armstrong (1964) demonstrated that 5-hydroxytryptamine (5-HT) has the ability to cause pain when applied to a blister base, and 5-HT was later shown to lower thresholds for chemically-induced pain in man (Sicuteri *et al.*, 1965) and to enhance pseudoaffective responses to bradykinin in animals (Nakano & Taira, 1976). Sensory nerve endings associated with small myelinated and non-myelinated axons have been found to be activated and sensitized by 5-HT in skin (Fjallbrant & Iggo, 1961; Beck & Handwerker, 1974) and muscle (Mense, 1981), as are cutaneous SAII mechanoreceptors with rapidly conducting afferent fibres (Fjallbrant & Iggo, 1961).

Although the pain evoked by application of 5-HT to a blister base has been demonstrated to be antagonized by ICS 205-930, and therefore probably involves a 5-HT_3-receptor (Donatsch *et al.*, 1984; Richardson *et al.*, 1985), in most cases the pharmacological identity of the 5-HT-receptor associated with sensory endings has not been established. The present study was undertaken to examine the effects of 5-HT on sensory receptors in the rat ankle joint, and to characterize the 5-HT receptors mediating these effects by the use of selective antagonists (Fozard, 1984; Bradley *et al.*, 1986; Brittain *et al.*, 1987). We also investigated whether 5-HT plays a role in the sensitization of high threshold mechanoreceptors in arthritic joints by using a rat model of adjuvant-induced mono-arthritis in which the arthritis is mild and confined to one ankle (Grubb *et al.*, 1988).

Methods

Induction of arthritis

Male Wistar rats weighing 200–250 g were anaesthetized with ether during subdermal injection of 0.15 ml of Freund's complete adjuvant (1.0 mg ml^{-1} heat killed *Myobacterium tuberculosis* in paraffin oil, Sigma) around the left ankle joint.

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Experiments were performed on anaesthetized animals following a period of two to nine weeks, during which time a localized arthritis consisting of swelling (approx. 50% increase in circumference of the left ankle joint) and redness of the injected ankle had developed and was maintained.

Surgical procedures

A total of 10 arthritic and 12 normal male Wistar rats weighing between 200 and 300 g was used in these experiments. Animals were anaesthetized with urethane (25% w/v, 0.6 ml $100\,g^{-1}$ body wt. i.p.). The trachea was cannulated and arterial blood pressure monitored via a cannula in the left carotid artery. A cannula was also inserted into the right femoral artery for the injection of drugs into the abdominal aorta at the level of the iliac bifurcation. Drugs were dissolved in 0.9% w/v aqueous NaCl solution (saline) and injected in volumes of 0.1 ml followed by a 0.2 ml saline wash. Accessibility to articular receptors via the vasculature was tested by use of a single low dose of capsaicin (1 μM i.a.) which caused a transient increase in neural discharge of all the afferents studied.

Electrophysiological recording

Neural recordings were made from the primary articulocutaneous ramus (PACR) of the left tibial nerve with platinum-iridium wire electrodes, by employing techniques described in detail elsewhere (Guilbaud *et al.*, 1985). Spontaneously active units for which no mechano-sensitive receptor fields could be found (termed 'chemosensitive' because of their excitation by 5-HT and capsaicin) and high threshold slowly-adapting mechanoreceptors with axons in the PACR were examined. Neural recordings were stored on videotape for subsequent analysis of individual afferent units by use of a pulse height voltage discriminator linked to a microcomputer.

Mechanoreceptors were identified by their response to mechanical stimulation, by use of smooth tipped glass probes of 0.5–1.0 mm diameter. Thresholds of individual units were determined with a series of calibrated Von Frey hairs; these high threshold mechanoreceptors probably function as nociceptors (Wyke, 1981; Guilbaud *et al.*, 1985). Mechanical stimuli in any given trial were delivered at fixed intervals by an electromechanical indentation generator; ramp and plateau waveforms were used routinely, with indentations of 200–600 μm and 2 s duration repeated at 60–120 s intervals. The indentation probe consisted of a sealed metal tube (1 mm diameter) smoothed over at the tip with epoxy-resin, containing a silver wire core, insulated except at the tip, used as the cathode for localized electrical stimulation when measuring conduction velocities. Afferent fibre conduction velocity was measured by localized electrical stimulation, via the probe at the level of the receptor, and determining the time take for the action potential to reach the recording electrodes.

Data analysis

Neural discharge (counts per second) was plotted against time for each test, and the change in frequency from the pre-injection control period calculated. In order to standardize results from experiments with different absolute discharge frequencies, mean values for blocks of 10 s duration were used to calculate peak response as a percentage of the mean discharge in the pre-injection 10 s control period. Mechanoreceptor responses were quantified in counts per mechanical stimulus and expressed as a percentage of the pre-injection response.

Statistics

Mean values are given $\pm$ s.e.mean. Statistical analysis of differences between means was carried out by the Wilcoxon two-sample test (two-tailed) and the null hypothesis rejected if $P < 0.05$.

Drugs

The following compounds were used in this study, with concentrations being expressed in terms of the salt: 5-hydroxytryptamine creatinine sulphate complex, dopamine hydrochloride (Sigma); MDL 72222 ((1αH, 3α, 5αH-tropan-3-yl) 3,5-dichlorobenzoate methanosulphonate salt, kindly donated by Merrell Dow Research Institute Strasbourg); ICS 205-930 ((3α-tropanyl)-3yl)-1H-indole-3-carboxylic acid ester, kindly donated by Sandoz, Basel); GR 38032F (1,2,3,9-tetrahydro-9-methyl-3-[(2methyl-1H-imidazol-1-yl)methyl]-4H-carbazol-4-one hydrochloride dihydrate, kindly donated by Glaxo Group Research, Ware); ketanserin, tartaric acid salt (kindly donated by Janssen Pharmaceuticals, Beerse). Capsaicin (8-methyl-n-vanylyl-nonenamide, Sigma) was prepared by diluting a stock solution (1 mg ml^{-1} in 10% ethanol: 10% Tween 80: 80% saline) in saline.

Results

Two types of sensory activity were investigated: mechanoreceptors for which receptive fields were found in the joint capsule (Guilbaud *et al.*, 1985), and 'chemosensitive' units, previously described by Grubb *et al.* (1988), which were excited by 5-HT and capsaicin but for which no receptor fields for mechanical stimuli were found (Table 1). Both mechanosensitive and chemosensitive units were excited by capsaicin (1–10 μg, i.a.) in all experiments. Low threshold, rapidly adapting mechanoreceptors with receptive fields in the tissues adjacent to the joint capsule were not considered in these studies.

Normal rats – mechanoreceptors

Mechanoreceptors with afferent fibres in the PACR and with receptive fields in the ankle joint tissues of normal rats had high mechanical threshold, were slowly adapting with punctate receptive fields of approximately 1 mm diameter and were therefore similar to those described by Guilband *et al.*, (1985). Units had mean von Frey thresholds of 81 $\pm$ 6.8 mN, and the conduction velocities of their afferents (2.1–10.5 ms^{-1}) indicated that they were C or Aδ fine afferent fibres. Only one mechanosensitive unit showed any background (spontaneous) discharge (0.2 i.p.s.) before the addition of 5-HT – the number of these high threshold units found in individual animals was small, and their lack of resting discharge and high mechanical

Table 1 Summary of the number of mechanosensitive and chemosensitive units responding to 5-hydroxytryptamine (5-HT) and the minimal effective doses for these effects in normal and arthritic rats

			No. of units displaying each type of response		
	Units	n	*Rapid excitation*	*Delayed response*	*Minimal effective dose* (μg)
Normals	Mechanosensitive	6	4	3	100
	Chemosensitive	16	12	12	1
Arthritic	Mechanosensitive	10	3	5	1
	Chemosensitive	12	7	12	1

thresholds made them difficult to find. A summary of all units responding to 5-HT is given in Table 1.

Effects of 5-HT on responsiveness to mechanical stimulation In the six units examined 5-HT (1–100 μg) evoked a dose-dependent increase in responsiveness to the standard mechanical stimulus (illustrated for 100 μg in Figure 1). The minimal effective dose which gave reproducible responses in all four of the units tested in this way was found to be 5 μg. As illustrated in Figure 2 a mean peak increase of 56% ($n = 4$) in response to subsequent mechanical stimuli was observed following injection of 5 μg 5-HT, and this effect lasted for 38 $\pm$ 34 s. Larger doses of 5-HT had a more prolonged action, as can be seen in Figure 1 where the response to a mechanical stimulus was still elevated three minutes after the injection of 100 μg 5-HT. Repeated injections of 5-HT produced a sensitization to the drug in four mechanosensitive units.

Excitatory effects of 5-HT on spontaneous activity of mechanosensitive units Close arterial injection of 1–100 μg 5-HT evoked a discharge in three previously silent mechanoreceptors and enhanced the discharge of one unit from a very low initial level of discharge. The effect was reproducible in two of these units at the highest dose used (100 μg 5-HT). A biphasic response observed following injection of 5-HT consisted of a transient burst of activity (hereafter called a 'fast' response) with rapid onset (<10 s), followed by a delayed (>20 s), longer-lasting increase in discharge, hereafter called a 'slow' response (see Figure 3).

Normal rats – chemosensitive afferents

In the 12 normal animals examined in this study 16 recordings consisting of between one and three different action potential spikes were obtained from units with a low level of activity before the addition of 5-HT. Their action potential spike characteristics were similar to those of identified C-fibre afferents, and their mean rate of discharge was 1.4 $\pm$ 0.3 i.p.s.

Excitatory effects of 5-HT on chemosensitive units All the units with an ongoing discharge were excited by an initial or subsequent injection of 5-HT (1–100 μg, i.a.); a further three units became spontaneously active following the drug administration.

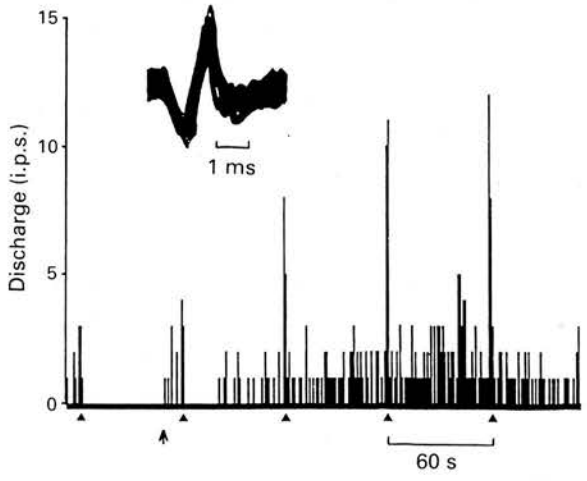

Figure 1 Effects of 100 μg 5-hydroxytryptamine (5-HT) on the activity of a high threshold mechanoreceptor with afferent fibre conduction velocity of 4 ms^{-1} from a normal animal. The graph (bin width 1 s) illustrates afferent discharge and shows fast and slow excitation. Mechanical stimuli (arrowheads) were repeated once every minute and neural responsiveness was increased following an injection of 5-HT (arrow). The inset shows 100 superimposed oscilloscope sweeps of the single afferent unit whose discharge was counted.

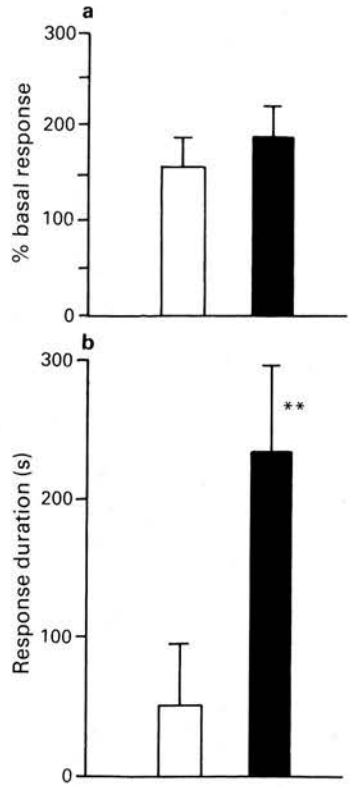

Figure 2 Effects of 5-hydroxytryptamine (5-HT) on mechanoreceptor responsiveness in both normal and arthritic rats. (a) The mean peak increase in mechanoreceptor responsiveness is shown as a percentage of preinjected control produced by 5 μg 5-HT (i.a.) in four normal rats (open column) and by 1 μg 5-HT (i.a.) in six arthritic rats (solid column). (b) Illustrates mean duration of increased responsiveness produced by the same injections of 5-HT (normal rats, open column; arthritics, solid column). Bars represent s.e. mean. ** $P < 0.01$ (Wilcoxon, two-tailed).

Two main components could be recognised in the response to 5-HT. An early, brief burst of activity, which was seen in 75% of active units, followed by a slow sustained increase in discharge in 75% of units. Responses in individual units were either monophasic or biphasic; fast responses occurred within 10 s following injection of 5-HT and lasted for a maximum of 30 s. Tachyphylaxis developed to repeated injections of 5-HT (20–100 μg at 10 min intervals; data not illustrated). However, with lower doses (1–10 μg) and a 15 min interval between injections, a relatively consistent response was obtained in four recordings. The slow response generally took longer than 15 s to develop and lasted for over 4 min in some cases. Depression of activity following the initial excitation was also seen in a small number of units when background activity was elevated.

Arthritic rats – mechanoreceptors

A characteristic feature of arthritic preparations, as previously described (Guilbaud *et al.*, 1985), was the large number of mechanoreceptors found in the joint. On average approximately three times as many mechanoreceptors were found in the ankle joint tissues of arthritic rats in comparison with control animals. These units had lower thresholds than normal for activation with von Frey hairs (52.7 $\pm$ 4.6 mN, $P < 0.05$) and generally had overlapping receptive fields; their conduction velocities (0.5–7.8 ms^{-1}) indicated that they were C/Aδ fibres. In contrast to the lack of spontaneous mechanoreceptor activity in normal rats, nine of the 10 mechanosensitive units studied showed spontaneous discharge, averaging 1.2 $\pm$ 0.4 i.p.s., before the administration of 5-HT.

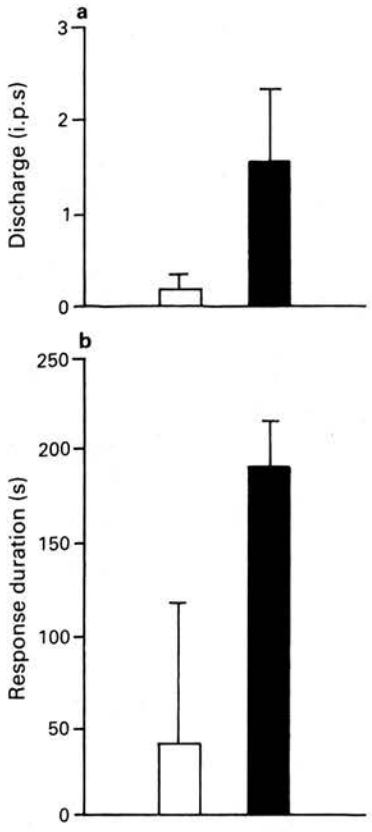

Figure 3 Comparison of 5-hydroxytryptamine (5-HT)-induced slow excitation of mechanosensitive units in normal and arthritic rats. (a) Shows the peak increase in discharge produced by 100 μg 5-HT (i.a.) in two responding units of six tested from normal rats (open column) and the mean peak increase produced by 1 μg 5-HT in four out of five units from arthritics (solid column). The mean basal rate of discharge in arthritic rats was 1.1 ± 0.8 i.p.s., whereas in control rats no activity was present before the injection of 5-HT. (b) Shows the duration of effects for the same injections as in (a). Open column, represents data from the two individual units which were excited by 5-HT in normal rats. Solid column represents mean of four units from arthritic rats. Bars show s.e.mean.

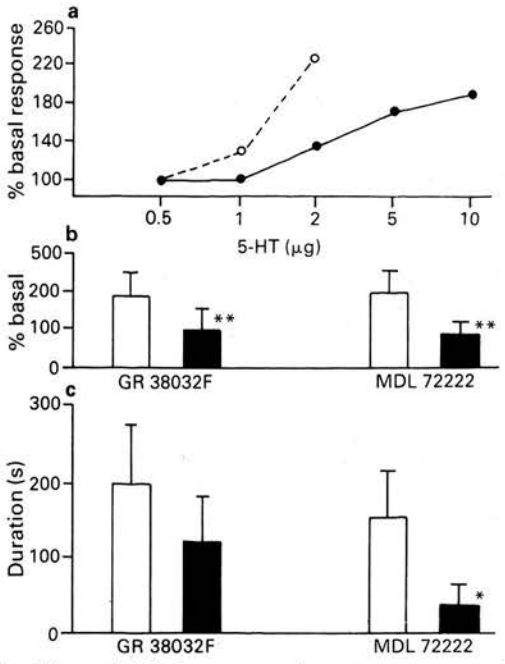

Figure 4 Effects of 5-hydroxytryptamine$_3$ (5-HT$_3$)-antagonists GR 38032F (100 μg kg^{-1}, i.a.), MDL 72222 (100 μg kg^{-1}, i.a.) and ICS 205-930 (100 μg kg^{-1} i.a.) on 5-HT-induced enhancement of mechanoreceptor responsiveness in both normal and arthritic rats. (a) Shows a shift to the right of the log dose-response curve caused by ICS 205-930 (100 μg kg^{-1} i.a.) in a high threshold mechanoreceptor with afferent fibre conduction velocity of 2.7 ms^{-1} from an arthritic rat. (○) and (●) responses before and after addition of antagonist, respectively. The dose of 5-HT is shown in μg with the peak response obtained given as a percentage of the preinjection control. (b) Illustrates the effect of GR 38032F ($n = 5$) and MDL 72222 ($n = 5$) on the mean peak increase in mechanoreceptor responsiveness produced by an effective standard dose of 5-HT (1–100 μg i.a.). Columns represent peak responses before (open) and after (solid) injection of antagonist. (c) The duration of response obtained for the same injections as in (a). Bars represent s.e.mean. Significantly different mean values are shown as: * $P < 0.05$ and ** $P < 0.01$.

Effects of 5-HT on responsiveness to mechanical stimulation In all 10 units examined a dose-dependent increase in responsiveness to the standard mechanical stimulus was obtained following the injection of 5-HT (1–100 μg). In seven units the effective threshold dose for production of consistent responses was found to be 1 μg. A mean increase of 75% ($n = 6$) in response to subsequent mechanical stimuli was produced following injection of the threshold dose. This effect lasted for 240 ± 48 s, a duration which is significantly greater ($P < 0.01$) than that produced by 5 μg 5-HT in normal rats (Figure 2). Sensitization of mechanoreceptor responses to 5-HT was observed in two units.

Excitatory effects of 5-HT on activity of mechanosensitive units Increases in spontaneous activity of six mechanosensitive units was seen following injection of 5-HT (1–100 μg). The biphasic response seen in normal animals was much less conspicuous, being obtained in only one recording. One unit gave a fast and slow response, and another unit displayed only the fast response. Three units responded with only a slow increase in spontaneous activity. In four of the units the slow response consisted of a mean peak increase of 1.6 ± 0.6 i.p.s. above basal discharge (1.1 ± 0.7 i.p.s.) which lasted for 185 ± 34 s. This effect of 5-HT contrasts markedly with the small response obtained following injection of 100 μg 5-HT in the normal rat (Figure 3). Spontaneous activity in a seventh unit was depressed following 5-HT administration.

Arthritic rats – chemosensitive units

Chemosensitive units Spontaneously active units lacking any identifiable mechanosensitive receptive fields were more numerous in arthritic rats than in controls. In experiments on 10 animals the effects of 5-HT on spontaneous discharge were examined in 12 recordings consisting of between one and three different units with action potentials characteristic of identified C-fibre afferents. Their mean rate of spontaneous discharge before the administration of 5-HT was 1.4 ± 0.2 i.p.s.

Excitatory effects of 5-HT on chemosensitive units All the units examined were responsive to injections of 5-HT (1–100 μg). A biphasic response was seen, as in normal rats. A fast excitatory response was seen in 58% of units, followed in all the units studied by a slow long lasting increase in discharge.

Effects of 5-HT-receptor antagonists in normal and in arthritic rats

The 5-HT receptor antagonists, MDL 72222, ICS 205-930 and ketanserin, were administered at 100 μg kg^{-1} i.a., doses previously found to be active in abolishing chemoreceptor responses to 5-HT in the cat (Kirby & McQueen, 1984). In the present experiments the 5-HT$_3$-receptor antagonists selectively blocked the 5-HT$_3$-receptor-mediated Bezold-Jarisch-like reflex evoked by 5-HT, and ketanserin selectively antagonized 5-HT-induced hypotension.

Table 2 Summary of the effects of 5-hydroxytryptamine (5-HT)-receptor antagonists on spontaneous discharge of chemosensitive units

	MDL 72222 ($100\,\mu g\,kg^{-1}$)		*ICS 205-930* ($100\,\mu g\,kg^{-1}$)		*GR 38032F* ($100\,\mu g\,kg^{-1}$)		*Ketanserin* ($100\,\mu g\,kg^{-1}$	
	n	% reduction in discharge	n	% reduction in discharge	n	% reduction in discharge	n	% reduction in discharge
Normals	4/4	53 (43–68)	3/3	62 (33–100)	4/4	53 (64–99)	5/9	63 (43–86)
Arthritic	1/4	100			4/4	36.5 (13–50)	6/9	58 (76–25)

Mean values are given – figures in parentheses show range of effect.
n = number of units.

Mechanoreceptor responsiveness

The 5-HT_3-receptor antagonists, MDL 72222, ICS 205-930 and GR 38032F, administered intra-arterially, each antagonized the 5-HT-induced sensitization of mechanoreceptors to mechanical stimuli in both normal and arthritic rats. In five units, treatment with MDL 72222 ($100\,\mu g\,kg^{-1}$) markedly reduced the increased responsiveness produced by 5-HT; injection of ICS 205-930 ($100\,\mu g\,kg^{-1}$) produced a clear rightward shift in the 5-HT dose-response curve in one unit. In studies on five units, GR 38032F ($100\,\mu g\,kg^{-1}$) abolished the response in one unit and produced a marked reduction in the response in the other four units (see Figure 4).

The 5-HT_2-receptor antagonist, ketanserin ($100\,\mu g\,kg^{-1}$), did not affect the 5-HT-evoked increase in mechanoreceptor responsiveness, either in normal or in arthritic rats when tested in six units.

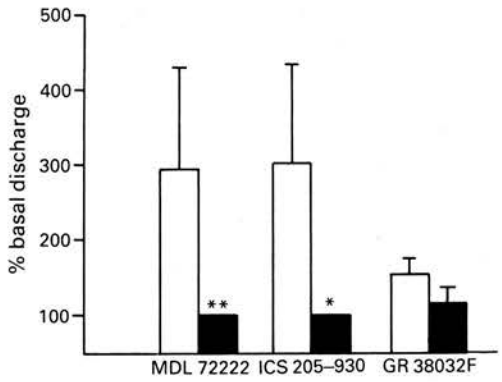

Figure 5 Effects of MDL 72222 ($100\,\mu g\,kg^{-1}$ i.a., $n = 6$), GR 38032F ($100\,\mu g\,kg^{-1}$ i.a., $n = 4$) and ICS 205-930) ($100\,\mu g\,kg^{-1}$ i.a., $n = 4$) on 5-hydroxytryptamine (5-HT)-induced fast excitation in chemosensitive units from both normal and arthritic rats. Each column shows the mean peak response as a percentage of basal discharge to a standard effective dose of 5-HT (5–$100\,\mu g$ i.a.) before (open) and after (solid) injection of antagonist. Bars represent s.e.mean. * $P < 0.05$ and ** $P < 0.01$.

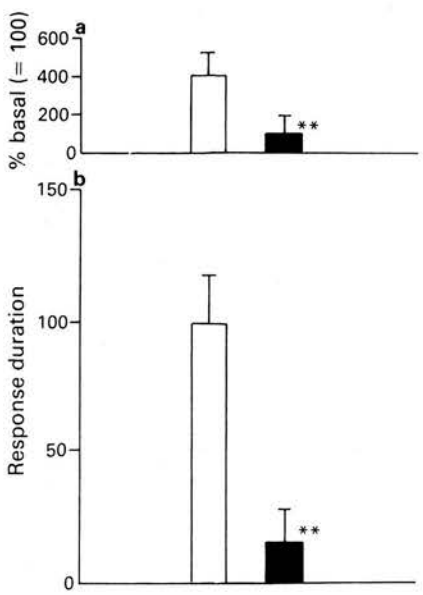

Figure 6 Effects of ketanserin ($100\,\mu g\,kg^{-1}$ i.a.) on 5-hydroxytryptamine (5-HT)-induced slow excitation of chemosensitive afferent units from normal and arthritic rats. (a) The mean peak discharge expressed as a percentage of preinjection control produced in response to a standard dose of 5-HT (1–$100\,\mu g$ i.a.) before (open column) and after (solid column) injection of ketanserin ($100\,\mu g\,kg^{-1}$, i.a., $n = 14$). (b) The mean duration of the effects shown in (a). Bars represent s.e.mean. ** $P < 0.01$.

Spontaneous activity of mechanosensitive units

In spontaneously active mechanosensitive units from arthritic rats, MDL 72222 ($100\,\mu g\,kg^{-1}$, $n = 1$) or GR 38032F ($100\,\mu g\,kg^{-1}$, $n = 2$) caused reductions in ongoing activity of 70% and 65% respectively when injected on their own. Reductions of activity lasted for no longer than 5 min in each case. In two mechanosensitive units, ketanserin ($100\,\mu g\,kg^{-1}$) had no effect on spontaneous activity.

An examination of the effects of the various 5-HT receptor antagonists on the fast and slow components of 5-HT-induced increases in spontaneous activity was complicated by the inconsistent nature of the fast response and its marked susceptibility to tachyphylaxis. The slow response, however, was consistently observed in all cases, and in arthritic animals neither MDL 72222 ($100\,\mu g\,kg^{-1}$, $n = 1$), ICS 205-903 ($100\,\mu g\,kg^{-1}$, $n = 1$) nor GR 38032F ($100\,\mu g\,kg^{-1}$, $n = 1$) had any affect on it. In only one out of two units did ketanserin cause a shift to the right of the 5-HT dose-response curve.

Chemosensitive units

In the case of spontaneously active chemosensitive units, MDL 7222, ICS 205-930 and GR 38032F all reduced ongoing discharge in arthritic rats as well as in normal animals previously exposed to 5-HT (see Table 2). Reductions in activity produced by antagonists lasted for 1–3 min. Ketanserin also markedly reduced ongoing discharge in arthritic and normal rats (Table 2).

Analysis of the effects of 5-HT-receptor antagonists on the fast response to injection of 5-HT were complicated by inconsistency of the response and its susceptibility to tachyphylaxis. However, it was markedly reduced or abolished following injections of the 5-HT_3-receptor antagonists MDL 72222 ($n = 6$), ICS 205-930 ($n = 4$) and GR 38032F ($n = 4$) (see Figure 5).

The slow response to injection of 5-HT was unaffected by the 5-HT_3-receptor antagonists, but in 14 out of 15 recordings was blocked or markedly reduced by ketanserin ($100\,\mu g\,kg^{-1}$) (see Figure 6).

Discussion

This investigation has shown that exogenous 5-HT can both excite and increase the responsiveness of sensory receptors

with fine (C, possibly some Aδ) afferents located in the ankle joint tissues of normal and arthritic rats. High threshold nociceptive mechanoreceptors were affected by 5-HT in both normal and arthritic animals, the effect of the amine on arthritic joints being more marked. The units recorded from arthritic joints had spontaneous activity, in contrast with the general lack of activity in units from normal joints. These findings are in good agreement with those obtained previously for the inflamed ankle joint (Guilbaud *et al.*, 1985). Furthermore, background discharge originating from chemosensitive units for which no mechanosensitive receptive fields could be found was greater in arthritic rats. These units were activated by 5-HT or capsaicin and responded to 5-HT with two components – fast brief excitation followed by a slow prolonged increase in activity. The effects of exogenous 5-HT on joint sensory receptors are quire similar to those on cat carotid chemosensors, where fast and slow excitatory effects involving 5-HT_3- and 5-HT_2-receptors, respectively, have been obtained (Kirby & McQueen, 1984).

Action of 5-HT on mechanoreceptors

Single close-arterial bolus injections of 5-HT increased the responses of high threshold mechanoreceptors to a standard mechanical stimulus for as long as six minutes. This duration of action is similar to that obtained for 5-HT-induced sensitization of high threshold mechanoreceptors in muscle to excitation induced by bradykinin (Mense, 1981), as well as for the action of 5-HT on SAII cutaneous mechanoreceptors (Fjallbrant & Iggo, 1961). MDL 72222, ICS 205-930 or GR 38032F prevented this action, but did not otherwise affect the response to mechanical stimuli. Ketanserin was without effect. These results suggest that the sensitization demonstrated may involve the action of 5-HT at a 5-HT_3-receptor located on the mechanoreceptor terminals within the joint tissues.

Fast excitation

Brief excitation of mechanosensitive units and chemosensitive units occurred within 10 s of the injection of 5-HT in both normal and arthritic joints. This action was blocked or reduced by the 5-HT_3-receptor antagonists. Fast depolarization evoked by 5-HT has been observed in several isolated neuronal preparations. For example, in cat and rabbit superior cervical ganglion (Haefely, 1974; Wallis & North, 1978), rabbit nodose ganglion (Higashi & Nishi, 1982) and guinea-pig coeliac ganglion (Wallis & Dun, 1988) 5-HT produced a rapid depolarization which was prone to tachyphylaxis and was sensitive to MDL 72222 or ICS 205-950 (Azami *et al.*, 1985; Round & Wallis, 1986; 1987; Wallis & Dun, 1988).

Slow excitation

The most consistent response to 5-HT was a slow dose-dependent long-lasting increase in discharge that was seen in the majority of the chemosensitive units examined, as well as in mechanosensitive units from normal and arthritic rats. The 5-HT_3-receptor antagonists had no effect on this slow excitation, whereas in the case of chemosensitive units ketanserin reduced or abolished it. The delayed nature of this effect could mean that 5-HT is acting indirectly to increase afferent activity. In our preparation, slow excitation was dose-dependent and outlasted the hypotensive effect of 5-HT, thus making it unlikely to be secondary to changes in blood pressure. Alternative mechanisms could include the involvement of a second messenger system in the afferent nerve terminal or the release of other algogenic substances from surrounding tissues by 5-HT. Evidence for a direct effect is suggested from studies on isolated neuronal preparations where a slow response produced by 5-HT has also been described (Kiraly *et al.*, 1983; Dun *et al.*, 1984).

The finding that long-lasting mechanoreceptor sensitization involves a 5-HT_3-receptor, whereas delayed excitation does not, suggests that separate mechanisms may be involved in receptor sensitization and 5-HT-induced excitation. This may relate to differing transduction pathways for mechanically- or chemically-evoked activation of sensory nerve endings.

Involvement of 5-HT in sensitization of sensory receptors during inflammation

The ability of 5-HT to sensitize high threshold articular mechanoreceptors suggests that endogenous 5-HT could play a role in the increased responsiveness of these receptors in chronically inflamed joints. Our results indicate that a 5-HT_3-receptor may be involved in this process. However, in arthritic rats the administration of antagonists selective for 5-HT_3- and 5-HT_2-receptors did not reduce mechanoreceptor sensitivity significantly, which they should have done if endogenous 5-HT acting at these receptors was a significant cause of sensitization. Low levels of spontaneous activity in mechanosensitive units recorded from arthritic joints were, on the other hand, reduced markedly by the addition of 5-HT_3-receptor antagonists. Similar results were obtained for chemosensitive units following administration of both 5-HT_3- and 5-HT_2-receptor antagonists. These observations suggest that while endogenous 5-HT may contribute to ongoing neural activity seen in inflamed joints, it is not a major factor in the sensitization of afferents.

In mechanoreceptors recorded from arthritic joints, with already enhanced mechanosensitivity, responsiveness to 5-HT was much greater than in normal joints, providing clear evidence that sensitivity of these sensors to 5-HT is increased in inflamed joints, and showing that the acute release of endogenous 5-HT could further boost sensitivity. The induction of high threshold mechanoreceptor sensitization and fast excitation by 5-HT, via a 5-HT_3-receptor, is consistent with the observation that pain produced by application to 5-HT to a blister base is mediated through a 5-HT_3-receptor (Donatsch *et al.*, 1984; Richardson *et al.*, 1985), assuming that the discharge recorded from the ankle joint afferents is involved in nociception. A role for 5-HT_2-receptors in 5-HT-induced pain has not previously been described, and further studies may be warranted in view of our results showing that 5-HT_2- and 5-HT_3-antagonists reduced afferent discharge in arthritic rats.

Finally, a role for 5-HT in the development of acute inflammatory pain has been suggested by Eschalier *et al.* (1989), who have shown that the administration of ICS 205-930 inhibits and reverses carrageenan-induced hyperalgesia in rats. It may be that endogenous 5-HT, released from platelets (Page, 1988), mast cells (Johnson & Erdos, 1973) or nerve fibres (Williams, 1967; Verhofstad *et al.*, 1981) is responsible for development of sensitization during the acute inflammatory response and become less important for chronic sensitization. However, further (acute) release of 5-HT may cause additional short-lasting sensitization of sensory receptors in chronic arthritis.

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A study of 5-HT-receptors associated with afferent nerves located in normal and inflamed rat ankle joints

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Abstract

Neural recordings were made from sensory fibres in a nerve supplying the ankle joint in normal rats and in rats with a novel monoarticular arthritis. The responses of mechanically and chemically sensitive units to intra-arterial injections of 5-HT were measured. In most cases the mechanosensitivity of sensory receptors in the ankle joint was not altered by 5-HT. However, 5-HT produced an increase in afferent activity in units which were identified as C-fibres on the basis of action potential amplitude and duration. The receptive fields of these chemosensitive units were not located. The responses of these units to 5-HT were dose dependent and were abolished by the 5-HT_2-antogonist, ketanserin, but not by the 5-HT_3-receptor antagonist, MDL 72222. The responses of chemosensitive units to injections of 5-HT were similar in normal and arthritic rats although the response was slightly prolonged in arthritic animals.

Introduction

The mechanical sensitivity of C- and Aδ-fibre joint afferents is increased in animals with inflamed joints (Guilbaud et al., 1985; Grigg et al., 1986) and this sensitisation can be reversed by administration of lysine acetylsalicylate (Guilbaud and Iggo, 1985) indicating that at least one group of the inflammatory mediators, the prostanoids, can sensitise afferent nerves or their receptors. Another agent released during tissue inflammation is 5-hydroxytryptamine (5-HT). In this study we have examined the effect of 5-HT on the discharge of afferent nerve fibres in normal and arthritic rat ankle joints.

Methods

1. Induction of arthritis. Rats were anaesthetised briefly with ether and an injection of 0.15 ml of Freund's complete adjuvant (Sigma Chem. Co. Ltd) was made into the tissues overlying the left ankle joint and the development of arthritis was followed over the next 4 weeks. Injections made in this way resulted in a localised arthritic lesion which predominantly involved the left ankle joint.

2. Neurophysiological recordings. Animals were anaesthetised with urethane (25%, 0.6 ml/100 g body weight) and the trachea and left carotid artery were catheterised. Blood pressure and respiration were monitored continuously. An additional catheter, used for injecting drugs, was inserted into the right femoral artery until the end was situated at the iliac bifurcation.

Neural recordings were made from the primary articulo-cutaneous ramus (PACR) using the techniques employed by Guilbaud et al. (1985). The responses of all units to injections of 5-HT (May and Baker Ltd) into the iliac bifurcation were ob-

served. All injections were made in a volume of 0.1 ml followed by a 0.2 ml saline wash. All numbers given are mean ± s.e.m. Data was analysed by playing recorded tapes through a CED interface (Cambridge Electronic Design Ltd) linked to a microcomputer. Most nerve strands contained between 1 and 4 active units and individual units were separated using a spike amplitude discriminator.

Results

1. General observations. Successful experiments were made on 9 rats, 5 arthritic (2–4 weeks post injection) and 4 controls and the physiological properties of control and inflamed joints were compared. Two major differences were observed. Firstly, the number of receptors which could be excited by mechanical stimulation was increased in inflamed joints compared to control joints indicating a lowering of the mechanical threshold of some of the mechanosensitive units. Secondly, spontaneously active units were present in 3 of 5 arthritic animals with an average of 3 active units in each case (mean discharge frequency = 0.6 ± 0.2 Hz) but were not recorded in control animals.

2. Injections of 5-HT. Following injections of 5-HT only two mechanosensitive units showed an increased response to graded mechanical stimulation. It was observed, however, that a population of afferent fibres which had previously been inactive responded to injections of 5-HT; it was not possible to identify their receptive fields by mechanical probing. The number of units responding to 5-HT was the same in control and arthritic animals. The majority of the action potentials recorded following an injection of 5-HT were extremely small and of long duration (2.6 ± 0.1 msecs) which is consistent with the activation of C-fibres by 5-HT in this preparation. The responses of these units to 5-HT were dose dependent with a threshold of 2–10 nmoles. A prolonged tachyphylaxis was observed if large doses (100 nmoles) were used.

There did not appear to be any difference in the sensitivity of the afferent units to 5-HT when normal and inflamed joints were compared and the mean latency of the responses to injections of 5-HT was similar (29.8 ± 8.3 secs). There was some evidence that the duration of the response to 5-HT was prolonged in the arthritic animals.

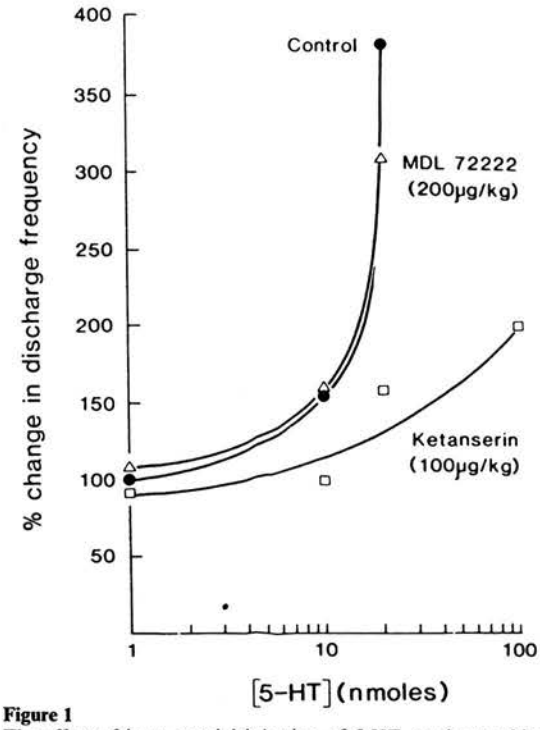

Figure 1
The effect of intra-arterial injection of 5-HT on the combined discharge frequency of 3 units in the PACR on a normal rat. ● = control injections of 5-HT, △ = injections of 5-HT following administration of 200 μg/kg MDL 72222, □ = injections of 5-HT following administration of 100 μg/kg ketanserin. MDL 72222 had no appreciable effect, whereas ketanserin, a 5-HT_2-receptor antagonist markedly reduced the 5-HT induced nerve activity.

3. Characterisation of the 5-HT-receptor. 5-HT is a potent algogen when applied to the blister base in man and it has been demonstrated that this effect is mediated through a 5-HT_3-receptor (Richardson et al., 1985). Following the identification of 5-HT sensitive units in each animal 100 μg/kg MDL 72222 (a 5-HT_3-receptor antagonist, Merrell Dow, Strasbourg) was given intra-arterially. MDL 7222 reduced the responses to 5-HT in only 1 of 4 control rats, and had no effect in 5 arthritic rats. Since MDL 72222 was generally ineffective in reducing the stimulation produced by 5-HT, 100 μg/kg ketanserin (a 5-HT_2-receptor antagonist), was given intra-arterially to each animal. In 3 of 4 control animals and in 4 of 5 arthritic animals the excitatory effects of 5-HT were markedly antagonised by this dose of ketanserin (Figure 1).

Discussion

An important issue in these experiments was whether it was appropriate to use this new model of arthritis for neurophysiological and neuropharmacological studies of inflamed joints. A comparison of the neurophysiological characteristics of sensory receptors in rats with fully developed arthritis (Guilbaud et al., 1985) and in rats with this new mild form of arthritis shows that they are almost identical.

In these experiments 5-HT was an excitatory agent for nerve fibres in the rat ankle joint, and its effects were predominantly mediated through a 5-HT_2-receptor. This finding is in contrast with the excitatory effects of 5-HT on the human blister base where 5-HT acts through the 5-HT_3-receptor to cause pain.

One major concern was that the excitatory effects of 5-HT might be secondary to drug-related changes in the local blood flow. This seems unlikely since vasoactive agents such as PGE_2 cause a systemic vasodilatation yet have no effect on sensory receptor discharge; also, there is evidence that blood flow in the hind-paw of the rat is unaffected by locally administered 5-HT (Owen, 1977).

A problem with these experiments is that the identity of the afferent units excited by 5-HT is uncertain since we were unable to locate their receptive fields by mechanical probing. However, it seems likely from analysis of spike width and spike height that they were C-fibres and could be involved in signalling noxious stimuli.

In summary, two important findings have arisen from these experiments. Firstly, it is possible using a low dose of F.C.A. to induce a localised inflammatory condition which appears, using neurophysiological criteria to induce a similar type of receptor sensitisation to that seen in rats with full polyarthritis. Secondly, 5-HT excites afferent fibres when injected into the ankle joint and this effect appears mainly to involve 5-HT_2-receptors.

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THE EFFECT OF PROSTAGLANDIN E_2 AND BRADYKININ ON THE DISCHARGE OF ARTICULAR MECHANORECPTORS IN NORMAL RATS.

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Afferent C-mechanoreceptors in the joint capsule of the rat ankle are sensitized in adjuvant induced polyarthritis (Guilbaud et al, Exp Brain Res 1985, 58:29). The responses of these receptors to graded mechanical stimuli are reduced in polyarthritic rats by the intravenous administration of lysine aspirin suggesting that prostaglandins are involved in the sensitization process (Guilbaud and Iggo, Exp Brain Res 1985, 61:164).

We have examined the effect of close arterial injections of PGE_2 (0.03-3 µg) on the responses of identified joint mechanoreceptors to graded mechanical stimuli in normal rats. In five anaesthetised rats, none of six units (2 A-delta and 4 C-fibre) was excited by PGE_2 and two units showed a significant depression (to 36% and 32% of control) at the highest dose (3 µg).

In the same experiments intra-arterial injections of threshold doses of the algogen bradykinin (0.01-10 µg) alone or in combination with PGE_2 (0.3-3.0 µg) were made. In 5/5 units the peak excitation elicited by the combination was larger than the response obtained with bradykinin alone. These results suggest that PGE_2 sensitises joint mechanoreceptors to bradykinin.

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Sensitization of joint capsule C- and A-δ high-threshold mechanoreceptors by 5-hydroxytryptamine (5-HT) in anaesthetized normal and arthritic rats

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Electrophysiological studies in rats with adjuvant-induced arthritis have shown that there is an increased sensitivity of joint capsule high threshold mechanoreceptors in the ankle joints of these animals compared with normal rats (Guilbaud *et al.* 1985). Investigations in our laboratory have shown that 5-HT produces excitation of C- and A-δ afferent fibres innervating rat ankle joint capsule receptors (Grubb *et al.* 1988). We now report further studies examining the effects of 5-HT on the responses of high threshold mechanoreceptors in the ankle joints of normal rats and those with unilateral adjuvant-induced chronic inflammation.

Male Wistar rats (200–300 g) were anaesthetized with urethane (25%, 0·6 ml 100 g^{-1}, I.P.). C- and A-δ fibre afferent discharge was recorded from the ankle joint in response to a standard mechanical stimulus, repeated every 1 or 2 min, using techniques described previously (Guilbaud *et al.* 1985).

Injection of 5-HT (1–100 μg, I.A.) produced a dose-dependent increase in response to the standard stimulus. In four recordings from normal animals, a mean increase of 116% (range: 17–333%, $n = 7$) in response to subsequent stimuli was observed following injection 5 μg 5-HT. Sensitization lasted for $69{\cdot}7 \pm 35{\cdot}4$ s (mean $\pm$ S.E.M.). In arthritic animals, six units displayed a mean increase of 71·1% (range: 15–180%, $n = 8$) in response to subsequent mechanical stimuli following injection of a correspondingly effective dose of 1 μg 5-HT. Sensitization lasted for $285 \pm 33{\cdot}8$ s.

Our results indicate that 5-HT sensitizes joint capsule C- and A-δ high threshold mechanoreceptors. In arthritic rats joint capsule receptors, which have an enhanced mechanical sensitivity, show greater than normal sensitivity to 5-HT.

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Actions of PGE_2 and cicaprost on the sensitivity of high-threshold mechanoreceptors in normal and inflamed ankle joints of the anaesthetized rat

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Lysine acetylsalicylate is able to reverse the sensitization of articular C-mechanoreceptors produced by chronic joint inflammation (Guilbaud *et al.* 1985; Guilbaud & Iggo, 1985). These experiments suggest that prostanoids may be involved in the sensitization of mechanoreceptors in inflamed joints. We have examined the action of PGE_2 and the stable PGI_2 analogue, cicaprost, on responsiveness of joint mechanoreceptors to mechanical stimuli in normal rats and in rats with modified adjuvant arthritis (Grubb *et al.* 1988).

Male Wistar rats were anaesthetized with urethane (25% w/v solution, 0·6 ml 100 g^{-1} body weight, I.P.) and the medial aspect of the left ankle joint was exposed. Nerve fibres isolated from the primary articulo-cutaneous ramus or tibial nerve were characterized by conduction velocity and a repeated graded mechanical stimulus was applied to the receptive field. Bolus injections of PGE_2 (0·03–30·0 μg) and cicaprost (0·01–2·0 μg) in normal saline were made through a femoral arterial cannula and lysine acetylsalicylate (50–100 mg kg^{-1}) was administered through a femoral venous cannula.

A total of 32 units were studied in 24 animals, 14 from arthritic and 18 from control animals. The responses of C- and A-δ high threshold mechanoreceptors to mechanical indentation of inflamed joints were measured before and after administration of salicylate, PGE_2 and cicaprost. In all units salicylate reduced the response to mechanical indentation. In 57% of units PGE_2 caused a partial reversal of the salicylate effect and in 43% there was no effect. Cicaprost caused a similar increase to PGE_2 in 83% of units and there was no effect in 17%. In normal animals PGE_2 caused excitation in 15%, had no effect in 54% and caused a depression in 31% of units. In contrast, cicaprost excited 54% of units and had no effect in 46%.

These results suggest that PGE_2 and cicaprost may act differentially to sensitize joint mechanoreceptors. This suggestion is supported by the observation that cicaprost and not PGE_2 induced spontaneous activity in units from both control and inflamed joints.

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Effects of aspirin and paracetamol on high-threshold tarsal joint mechanoreceptors in anaesthetized rats with adjuvant-induced arthritis

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In rats with adjuvant-induced polyarthritis the mechanical sensitivity of high-threshold slowly adapting mechanoreceptors (recorded from sensory nerve fibres innervating the tarsal joint) is reduced by lysine acetylsalicylate (Guilbaud & Iggo, 1985). The present experiments were undertaken to show whether aspirin has similar effects on these receptors in a model of adjuvant arthritis where the lesion is confined to one tarsal joint (Grubb *et al.* 1988). We also studied the effects of paracetamol so as to clarify the action of this drug on peripheral sensors.

Male Wistar rats were used; 0·15 mg Freund's complete adjuvant was injected, under halothane (2%) anaesthesia, around one tarsal (ankle) joint. Between 12 and 30 days later the animals were anaesthetized with urethane (25%, 0·6 ml 100 g^{-1} I.P.) and neural discharge from tarsal joint mechanoreceptors recorded (Grubb *et al.* 1988). The effects of I.V. lysine acetylsalicylate (in a dose equivalent to 50 mg kg^{-1} aspirin) or paracetamol (50 mg kg^{-1}) were studied on 'spontaneous' and on mechanically evoked discharge (400 μm indent, 2 s duration every 2 min – see Guilbaud & Iggo, 1985) and the conduction velocity measured. Results are shown in Table 1.

TABLE 1. Effects of aspirin and paracetamol (50 mg kg^{-1} I.V.) on activity ('spontaneous' and mechanically evoked) of tarsal joint mechanoreceptors in n arthritic rats

		n	Pre-drug discharge (100%)	Minimum discharge (%)	Time (min) to minimum
			Mean value ± S.E.M.		
Aspirin (L-ASA)	Spont. (ct/s)	4	2·5 ± 1·5	52 ± 8	16
	Mechn. (ct)	5	25 ± 4	65 ± 8	30
Paracetamol	Spont. (ct/s)	8	1·3 ± 0·4	66 ± 12	22
	Mechn. (ct)	4	35 ± 5	63 ± 24	18

Aspirin has similar effects in this preparation to those obtained by Guilbaud & Iggo (1985) – background and mechanically evoked discharge from mechanoreceptors, the majority of which had C fibre afferents, were both reduced by the salicylate. The effects of paracetamol on the receptors were not significantly different from those of aspirin ($P > 0·05$; Wilcoxon signed-ranks test).

These results support the proposal that prostanoids sensitize joint capsule mechanoreceptors in arthritis (Guilbaud & Iggo, 1985); they also provide direct evidence that paracetamol acts in the periphery to reduce input from these presumed nociceptors in adjuvant arthritis. Aspirin and paracetamol may act by a common mechanism, probably involving inhibition of cyclo-oxygenase.

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COMPARISON OF THE EFFECTS OF PGE2 AND CICAPROST ON THE RESPONSES OF RAT ANKLE JOINT MECHANORECEPOTORS TO BRADYKININ.

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Poster 222
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AIM OF INVESTIGATION: This study was undertaken to evaluate the effects of PGE_2 and cicaprost, a PGI_2 mimetic, on the responses of high threshold articular mechanoreceptors to bradykinin and mechanical stimuli.

METHODS: Male wistar rats were anaesthetized with urethane and recordings were made of A-delta and C-fibre afferent discharge from the ankle joint in response to a standard mechanical stimulus.

RESULTS: Close arterial injections of threshold doses of bradykinin (1-10ug) alone or in combination with either PGE_2 (0.3-3ug) or cicaprost (0.01-1.0ug) excited the receptors and also increased their responsivness to mechanical stimuli. In 8 of 10 units for PGE_2 and 7 of 8 units for cicaprost, the responses elicited by combined injections were were greater than those to either drug alone. Cicaprost and PGE_2 effects differed in that the former was a particularly potent excitant, producing long lasting periods of sustained activity in 6 out of 10 units.

CONCLUSIONS: These results, together with other studies, suggest that PGE_2 and cicaprost may act by different mechanisms to produce sensitization of joint mechanoreceptors to the actions of bradykinin.

Proceedings of Satellite Meeting to the VIth World Congress on Pain: The Neurobiology of Nociception, Kauai. 1990.

POSTER
2

COMPARISON OF THE EFFECTS OF PGE_2 AND CICAPROST ON THE RESPONSES OF RAT ANKLE JOINT SENSORY RECEPTORS TO BRADYKININ IN VITRO.

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AIM OF INVESTIGATION: This study was undertaken to evaluate the effects of PGE_2 and cicaprost, a PGI_2 mimetic, on the responsiveness of articular sensory receptors to bradykinin using a novel in vitro preparation.

METHODS: Male wistar rats were anaesthetized and following cannulation of the femoral artery for perfusion with oxygenated Krebs solution the left hindlimb was isolated in a superfusion bath at 30°C. Recordings were made of afferent discharge from the ankle joint in response to the application of drugs via the arterial perfusate.

RESULTS: Bradykinin (0.1-10ug) produced excitation when given alone or in combination with either PGE_2 (0.1-10ug) or cicaprost (0.01-1ug). In 4 of 8 recordings for PGE_2 and 10 of 14 for cicaprost, responses to combined applications were greater than those to either drug alone. Cicaprost proved to be more potent than PGE_2 in this respect and in contrast to PGE_2 was also a potent excitant when administered alone in 13 of 14 recordings. In all cases active units had action potential spike characteristics similar to those for identified A-delta and C-fibre afferents.

CONCLUSIONS: These results suggest that PGI_2 may be more effective than PGE_2 in producing the sensitizing effects of the protanoids on sensory receptors responding to noxious chemical stimuli.

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Effects of cicaprost and PGE_2 on the responses of rat ankle joint sensory receptors to bradykinin *in vitro*

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Recent studies on joint capsule sensory receptors have shown that the I.A. administration *in vivo* of cicaprost, a stable PGI_2 mimetic, and of PGE_2 can increase the responsiveness of these sensors to mechanical stimuli (Birrell *et al.* 1990). We now report the effects of cicaprost and PGE_2 on the receptors' responses to bradykinin (BK), using a novel *in vitro* preparation of the rat ankle (tibio-tarsal) joint.

Male Wistar rats (200–350 g) were anaesthetized with urethane (0·6 ml 25% w/v solution 100 g^{-1}, I.P.). The left femoral artery was cannulated to allow perfusion (2 ml min^{-1}) of the limb with Krebs solution (mM: NaCl, 118·4; KCL, 4·7; $MgSO_4.7H_2O$, 1·2; KH_2PO_4, 1·2; $NaHCO_3$, 25; $CaCl.2H_2O$, 2·5; glucose 11·1) equilibrated with 95% O_2 and 5% CO_2 at 32 °C, after heparinized saline (500 U kg^{-1} heparin) was flushed through the limb. Following surgical isolation of the limb, the paw was secured in a Perspex superfusion bath and the skin removed from the leg. Neural recordings were made as described previously (Guilbaud *et al.* 1985) from the desheathed nerve placed in a small side chamber filled with liquid paraffin. Drugs were injected into the perfusate.

For 14 high-threshold mechanoreceptors (nociceptors) tested, BK (0·1–10 μg) caused dose-dependent excitation in 9 instances (64%). Cicaprost (0·01–1 μg) evoked dose-dependent excitation in 11 of 12 (92%) recordings that was sustained for 10 min or more. PGE_2 (0·01–10 μg) only weakly and transiently excited 7 of 8 units (88%). Combined injections of threshold or subthreshold doses of cicaprost (0·01–1 μg and BK (0·01–10 μg) were more effective than either drug alone in 7 of 11 (64%) units. Combined injections of BK (0·1–10 μg) with PGE_2 (0·01–10 μg) produced enhanced excitation in only 3 of 7 (43%) recordings. These results provide further evidence to suggest that protanoids, and in particular PGI_2, increase the excitability of articular nociceptive sensory endings by a direct action in the periphery and also interact with BK. Furthermore, cicaprost on its own excited these receptors, suggesting that endogenous PGI_2 may directly activate articular nociceptors.

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Neuropeptide content of dorsal root ganglia cells in the arthritic rat

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It has been shown that the levels of certain neuropeptides in dorsal root ganglia (DRGs) and spinal cord are altered in experimental models of arthritis (Panerai *et al.* 1987; Kuraishi *et al.* 1989). The present study was undertaken to measure the neuropeptide content of DRGs at the C2 to T6 level of the spinal cord in rats with an adjuvant-induced monoarticular arthritis of the carpal joint.

Male Wistar rats (200–250 g) were divided into two groups of five. Under halothane anaesthesia, one group received an injection of 0·1 ml Freund's Complete Adjuvant (FCA, Sigma, 1 mg/ml) subdermally around the left carpal joint on Day 0, where as the other group was untreated and served as the control. There was an initial swelling of the joint in the FCA group which peaked at day 3 ($47{\cdot}7 \pm 4{\cdot}8\%$ increase in circumference compared to contralateral joint, $P < 0{\cdot}01$, Wilcoxon two sample test), followed by a chronic phase which peaked by day 15 ($26{\cdot}0 \pm 4{\cdot}3\%$ compared to contralateral joint, $P < 0{\cdot}01$).

Rats were killed by decapitation on Day 15 and Substance P (SP), calcitonin gene-related peptide (CGRP) and somatostatin (SST) content of DRGs from left and right sides in the regions C2 to T6 were measured by radioimmunoassay. Peptide levels were elevated the most at the C6 to C7 level, and are shown in Table 1.

Table 1. Levels of peptides in DRGs at the C6 to C7 level (pg/ganglion)

	Control		FCA	
	Left	Right	Left	Right
CGRP	$2914{\cdot}2 \pm 596{\cdot}6$	$2748{\cdot}3 \pm 336{\cdot}4$	$7831{\cdot}2^* \pm 106{\cdot}4$	$2374{\cdot}4 \pm 254{\cdot}7$
SP	$59{\cdot}4 \pm 5{\cdot}9$	$55{\cdot}9 \pm 4{\cdot}7$	$73{\cdot}0 \pm 7{\cdot}2$	$47{\cdot}2 \pm 7{\cdot}2$
SST	$46{\cdot}8 \pm 7{\cdot}3$	$43{\cdot}6 \pm 3{\cdot}6$	$49{\cdot}2 \pm 3{\cdot}1$	$39{\cdot}5 \pm 3{\cdot}0$

Data shown as mean ± S.E.M. $^*P < 0{\cdot}01$, compared to DRG on the right side.

In conclusion, CGRP content of DRGs from the area C6 to C7 were significantly elevated on the side ipsilateral to the arthritic carpal joint, whereas there was no significant difference in SST and SP content. This evidence suggests that CGRP in sensory neurons may be involved in the pathology of chronic inflammatory disease.

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